PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2001-136984
(43)Date of publication of application : 22.05.2001

(51)Int.Cl.

C12N 15/09
C12N 1/15
C12N 1/19
C12N 1/21
C12N 5/10
C12N 9/12
C12N 9/16
C12P 19/38
//(C12N 15/09
C12R 1:01)
(C12N 15/09
C12R 1:185)
(C12N 15/09
C12R 1:22)
(C12P 19/38
C12R 1:19)

(21)Application number : 2000-262120
(22)Date of filing : 31.08.2000

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(30)Priority
Priority number : 11249545 Priority date : 03.09.1999 Priority country : JP

(54) VARIANT NUCLEOSIDE-5'-PHOSPHATE-PRODUCTIVE ENZYME

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a new variant nucleoside-5'-phosphate-productive enzyme improved in nucleoside-5'-phosphate productivity, to provide a new means for obtaining the enzyme, and to provide uses of the above enzyme.
SOLUTION: This variant nucleoside-5'-phosphate-productive enzyme has such characteristics that there are one Lys residue, two Arg residues and two His residues, the Cα-Cα distances therebetween stand within a specific range, respectively, there is a space to which a nucleoside is bound in the proximity thereto, there have phosphate group transfer activity and/or phosphatase activity, and thereby nucleoside-5'-phosphate productivity is improved. A method for producing this enzyme by determining a variation based on the X-ray crystal structural analysis of the corresponding well-known enzyme is provided. By this method, the objective enzyme of higher activity can be easily obtained.

CLAIMS

[Claim(s)]
[Claim 1]In a nucleoside 5'-phosphoric acid production enzyme, one Lys residue, two Arg residue, Two His residue exists and there is distance between such Calpha within limits shown in drawing 1, And a variant nucleoside 5'-phosphoric acid production enzyme whose nucleoside 5'-phosphoric acid productivity of an enzyme which has a space which a nucleoside combines near the, and which has phosphoryl-group-transfer activity and/or phosphatase activity improved.
[Claim 2]The variant nucleoside 5'-phosphoric acid production enzyme according to claim 1 which is that to which the origin of an enzyme belongs to the Escherichia bacteria, the Morganella bacteria, Providencia bacteria, the Enterobacter bacteria, KUREBUJIERA group bacteria, or the Enterobacter bacteria.
[Claim 3]It carries out based on structure coordinates shown in atomic coordinate data produced by conducting X ray crystal structure analysis of the crystal of Escherichia BURATTAE origin acid phosphatase, The variant nucleoside 5'-phosphoric acid production enzyme according to claim 1 which presumed a bond form with nucleosides, such as inosine and guanosine, and a phosphoric acid compound of those and whose nucleoside 5'-phosphoric acid productivity improved by substitution, such as amino acid residue and/or a supplementary factor, an addition, and deletion.
[Claim 4]A following position of an amino acid sequence of Escherichia BURATTAE origin acid phosphatase (residue in Ser72 of Escherichia BURATTAE acid phosphatase, or Ser72 to 10A) : 16, 67-76, 78-79, 96, 99-100, 102-104, 106-108, 115 and 140, 148-154, a variant nucleoside 5'-phosphoric acid production enzyme that change has produced in at least one position of 157, 179, and 183.
[Claim 5]With an enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity, when amino acid sequence alignment with Escherichia BURATTAE origin acid phosphatase is carried out, A following position of an amino acid sequence of Escherichia BURATTAE origin acid phosphatase (residue in Ser72 of Escherichia BURATTAE acid phosphatase, or Ser72 to 10A) : 16, 67-76, 78-79, 96, 99-100, 102-104, 106-108, 115 and 140, 148-154, a variant nucleoside 5'-phosphoric acid production enzyme that change has produced in at least one position corresponding to 157, 179, and 183.
[Claim 6]With an enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity, when alignment with the three-dimensional structure of Escherichia BURATTAE origin acid phosphatase is performed by the TOREDDINGU method, A following position of an amino acid sequence of Escherichia BURATTAE origin acid phosphatase (residue in Ser72 of Escherichia BURATTAE acid phosphatase, or Ser72 to 10A) : 16, 67-76, 78-79, 96, 99-100, 102-104, 106-108, 115 and 140, 148-154, a variant nucleoside 5'-phosphoric acid production enzyme that change has produced in at least one position corresponding to 157, 179, and 183.
[Claim 7]The origin of an enzyme is of the Enterobacter aerogenes origin, and in the amino acid sequence The 14th leucine residue, The 61st leucine residue, the 63rd alanine residue, the 64th glutamate residue, The 67th asparagine residue, the 69th serine residue, the 70th alanine residue, The 71st glycine residue, the 72nd glycine residue, the 101st isoleucine residue, The 102nd glutamate residue, the 133rd threonine residue, the 134th glutamate residue, A variant nucleoside 5'-phosphoric acid production enzyme in which at least one amino acid residue is replaced by other amino acid

residue among the 138th leucine residue, the 149th threonine residue, and the 151st isoleucine residue.
[Claim 8]. Became final and conclusive from an enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity, or a spacial configuration produced by conducting X ray crystal structure analysis of the crystal of a complex of it and molybdic acid. A manufacturing method of a variant nucleoside 5'-phosphoric acid production enzyme manufacturing an enzyme variant whose nucleoside 5'-phosphoric acid productivity improved an active site of this enzyme, and/or amino acid residue which is in less than 10A from it by carrying out substitution, an addition, and deletion.
[Claim 9]How to use structure coordinates of Escherichia BURATTAE origin acid phosphatase, and to manufacture inhibitor of phosphatase or phosphotransferase.
[Claim 10]One crystal of the complexes of an enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity, or it and molybdic acid.
[Claim 11]A crystal of Escherichia BURATTAE origin acid phosphatase which has space group $P6_322$ of a hexagonal system.
[Claim 12]A crystal of Escherichia BURATTAE origin acid phosphatase G74 D/I153T enzyme variant which has space group $P2_12_12_1$ of an ortho rhombic system.
[Claim 13]A crystal of a complex (reaction intermediate analog) of Escherichia BURATTAE origin acid phosphatase and molybdic acid which have space group $P3_121$ of a trigonal system.
[Claim 14]A gene which encodes an enzyme of a statement in any 1 paragraph of claims 1-7.
[Claim 15]A recombinant DNA containing the gene according to claim 14.
[Claim 16]A microorganism which holds the recombinant DNA according to claim 15.
[Claim 17]An enzyme given in any 1 paragraph of claims 1-7, or a microorganism containing it, Or a manufacturing method of nucleoside 5'-phosphoric acid making the microorganism according to claim 16 act on a nucleoside and a phosphate donor, making nucleoside 5'-phosphoric acid generate, and extracting this.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates to a variant nucleoside 5'-phosphoric acid production enzyme whose nucleoside 5'-phosphoric acid productivity improved, and a manufacturing method for the same. This invention relates to enzymes useful to manufacture of the above-mentioned enzyme. This invention relates to the microorganism which holds the recombinant DNA containing the gene which encodes the useful aforementioned enzyme variant in the manufacturing method, and this gene, and this recombinant DNA, concerning the manufacturing method of nucleoside 5'-phosphoric acid. Nucleoside 5'-phosphoric acid is useful as a seasoning, medicines, and those raw materials. It is based on this invention having succeeded in the break through of the proteinic new spacial configuration by X ray crystal-structure-analysis art, and this spacial configuration has possibilities which are not restricted to a microorganism.
[0002]
[Description of the Prior Art]As a method of phosphorylating a nucleoside biochemically and manufacturing nucleoside 5'-phosphoric acid cheaply and efficiently, By making a specific microbial cell act on the phosphate donor chosen from the group which comprises a nucleoside and polyphosphoric acid (salt), phenylphosphoric acid (salt), and carbamyl phosphate under acid conditions, The method of generating nucleoside 5'-phosphoric acid efficiently is developed, without being accompanied by the byproduction of nucleoside 2'-phosphoric acid and a nucleoside 3'-phosphoric acid isomer (JP,7-231793,A). Then, the gene which encodes acid phosphatase is acquired from Escherichia BURATTAE (Escherichia blattae) and Morganella MORUGANI (Morganella morganii), It was checked by carrying out the extensive manifestation of this gene by Escherichia coli in gene engineering that the productivity of nucleoside 5'-phosphoric acid improves further. The structure of this acid phosphatase is shown in drawing 2. Drawing 2 Namely, Escherichia BURATTAE origin acid phosphatase. The amino acid sequence of (writing it as EB-AP hereafter) Morganella MORUGANI, Salmonella typhimurium (Salmonella typhimurium), They are an amino acid sequence of ZAIMO monas MOBIRISU (Zymomonas mobilis) origin acid phosphatase, and the aligned figure. The base sequence of the gene of each acid phosphatase and the amino acid sequence of the enzyme by which a code is carried out are shown in the array numbers 1-8 of an array table. An asterisk shows the saved residue by drawing 2. After aligning the field of secondary structure, it was shown with a stick. The portion enclosed with the line of the rectangular head shows the motif which is common between acid phosphatase families. The motif consists of three domains, the 1KXXXXXXRP array number 121, the 2PSGH array number 122, and 3SRXXXXXHXXXD array number 123**. Here, X is arbitrary amino acid. This acid phosphatase (drawing 2) has the superior phosphatase activity which decomposes nucleoside 5'-phosphoric acid into a nucleoside in a wild type although it has phosphoryl-group-transfer activity, and there was a fault into which the accumulated nucleoside 5'-phosphoric acid will be decomposed. Then, generate many enzyme variants at random and the variant acid phosphatase whose phosphoryl-group-transfer activity improved relatively as compared with phosphatase activity from the inside is found out, It was shown by by carrying out the extensive manifestation of this variant acid phosphatase gene that the productivity of nucleoside 5'-phosphoric acid improves by leaps and bounds (JP,9-37785,A). The compatibility of this variant phosphatase over a nucleoside is

improving.
It is thought that phosphoryl-group-transfer activity has been improved by that cause.
The above-mentioned Escherichia BURATTAE origin variant acid phosphatase (= G74 D/I153T enzyme variant), Although phosphoryl-group-transfer activity is weaker than G72 D/I151T enzyme variant in which Morganella MORUGANI origin acid phosphatase (MM-AP) corresponds, The 10 residue substitution L63 Q/A65 Q/E66 A/N69 D/S71 A/S72 A/G74 D/T135 K/E136 D/I153T enzyme variant which replaced eight amino acid residue by the amino acid of MM-AP corresponding on the primary structure (henceforth) It was only shown that 10 residue substitution variant EB-AP and description acquire phosphoryl-group-transfer activity almost equivalent to G72 D/I151T variant MM-AP (JP,10-201481,A).
[0003]
[Problem(s) to be Solved by the Invention]The G74 D/I153T variant enzyme gene of the Escherichia BURATTAE origin acid phosphatase (EB-AP) whose above-mentioned productivity improved, the method of producing nucleoside 5'-phosphoric acid is established by carrying out the extensive manifestation of the 10 residue substitution variant enzyme gene by Escherichia coli -- having had (JP,9-37785,A, JP,10-201481,A) -- variant EB-AP whose productivity improved is desired. This invention makes it a technical problem to aim at further improvement in nucleoside 5'-phosphoric acid productivity by designing variant EB-AP based on the three-dimensional structure of EB-AP.
[0004]
[Means for Solving the Problem]If this invention is outlined, it will be as enumerating below.
In a nucleoside 5'-phosphoric acid production enzyme, (1) One Lys residue, Two Arg residue and two His residue exist, and there is distance between such Calpha within limits shown in having [illegible], And a variant nucleoside 5'-phosphoric acid production enzyme whose nucleoside 5'-phosphoric acid productivity of an enzyme which has a space which a nucleoside combines near the, and which has phosphoryl-group-transfer activity and/or phosphatase activity improved.
(2) A variant nucleoside 5'-phosphoric acid production enzyme given in (1) paragraph which is that to which the origin of an enzyme belongs to the Escherichia bacteria, the Morganella bacteria, Providencia bacteria, the Enterobacter bacteria, KUREBUJIERA group bacteria, or the Enterobacter bacteria.
(3) It carries out based on structure coordinates shown in atomic coordinate data produced by conducting X ray crystal structure analysis of the crystal of EB-AP, A variant nucleoside 5'-phosphoric acid production enzyme given in (1) paragraph given nucleoside 5'-phosphoric acid productivity improved by presuming a bond form with nucleosides, such as inosine and guanosine, and carrying out substitution, such as amino acid residue and/or a supplementary factor, an addition, and deletion.
(4) . A following position of an amino acid sequence of EB-AP. amino acid Ser72 of EB-AP. Or residue in Ser72 to 10A :. 16, 67-76, 78-79, 96, 99-100, 102-104, 106-108, 115 and 140, 148-154, a variant nucleoside 5'-phosphoric acid production enzyme that change has produced in at least one position of 157, 179, and 183.
(5) With an enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity. When amino acid sequence alignment with EB-AP is carried out, A following position of amino acid sequence ** of EB-AP. amino acid Ser72 of EB-AP. Or residue in Ser72 to 10A :. 16, 67-76, 78-79, 96, 99-100, 102-104, 106-108, 115 and 140, 148-154, a variant nucleoside 5'-phosphoric acid production enzyme that change has produced in at least one position of 157, 179, and 183.
(6) With an enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity. When alignment with the three-dimensional structure of EB-AP is performed by the TOREDDINGU method, A following position of amino acid sequence ** of EB-AP. amino acid Ser72 of EB-AP. Or residue in Ser72 to 10A :. 16, 67-76, 78-79, 96, 99-100, 102-104, 106-108, 115 and 140, 148-154, a variant nucleoside 5'-phosphoric acid production enzyme that change has produced in at least one position of 157, 179, and 183.
[0005](7) The origin of an enzyme is of the Enterobacter aerogenes origin, In the amino acid sequence, the 14th leucine residue, the 61st leucine residue, The 63rd alanine residue, the 64th glutamate residue, the 67th asparagine residue, The 69th serine residue, the 70th alanine residue, the 71st glycine residue, The 72nd glycine residue, the 101st isoleucine residue, the 102nd glutamate

residue, The 133rd threonine residue, the 134th glutamate residue, the 138th leucine residue, A variant nucleoside 5'-phosphoric acid production enzyme in which at least one amino acid residue is replaced by other amino acid residue among the 149th threonine residue and the 151st isoleucine residue.
(8) An enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity, . Or became final and conclusive from a spacial configuration produced by conducting X ray crystal structure analysis of the crystal of a complex of it and molybdic acid. amino acid residue (constituted by Lys1 ** and two Arg(s) His two) located in an active site of this enzyme -- and/or -- and amino acid residue in less than 10A by carrying out substitution, an addition, and deletion, A manufacturing method of a variant nucleoside 5'-phosphoric acid production enzyme manufacturing an enzyme variant whose nucleoside 5'-phosphoric acid productivity improved.
(9) How to use structure coordinates of Escherichia BURATTAE origin acid phosphatase, and to manufacture inhibitor of phosphatase or phosphotransferase.
[0006](10) One crystal of the complexes of an enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity, or it and molybdic acid.
(11) A crystal of EB-AP which has space group $P6_322$ of a hexagonal system.
(12) A crystal of EB-AP G74 D/I153T enzyme variant which has space group $P2_12_12_1$ of an ortho rhombic system.
(13) A crystal of a complex (reaction intermediate analog) of EB-AP and molybdic acid which have space group $P3_121$ of a trigonal system.
[0007](14) A gene which encodes an enzyme of a statement in any 1 paragraph of (1) - (7) paragraph.

(15) A recombinant DNA which contains a gene of a statement in (14) paragraphs.
(16) A microorganism which holds a recombinant DNA of a statement in (15) paragraphs.
[0008](17) An enzyme given in any 1 paragraph of (1) - (7) paragraph, or a microorganism containing it, Or a manufacturing method of nucleoside 5'-phosphoric acid making a microorganism given in (16) paragraphs act on a nucleoside and a phosphate donor, making nucleoside 5'-phosphoric acid generate, and extracting this.
[0009]Based on the three-dimensional structure of EB-AP, this invention builds a bond form model with a nucleoside, and provides a production method of nucleoside 5'-phosphoric acid using variant EB-AP designed based on it.
[0010]
[Embodiment of the Invention]Hereafter, this invention is explained concretely.
(1) In order to determine the proteinic three-dimensional structure by X ray crystal structure analysis, it is necessary to crystallize protein (details were shown in Example 1-3). In order to crystallize protein, pH, the kind of buffer, the concentration of a buffer and the kind of precipitant, the concentration of a precipitant, the concentration of additive agents, such as metal, the concentration of protein, proteinic purity, etc. must determine many parameters by trial and error. Therefore, before obtaining a crystal, usually the time for several months - several years will be taken, and there is also a case where a crystal is not obtained against a great labor, plentifully. although crystallization is indispensable for three-dimensional structure determination -- other than this -- being also alike -- there are a purification method of a proteinic high grade, stable Conservation Act whose protease resistance it is high-density and is strong, and also usefulness industrial as a process before fixed use of an enzyme.
(2) Irradiate the produced crystal with X-rays and collect diffraction data. A crystal protein has plentifully a case where X-ray irradiation receives a damage and diffraction ability deteriorates. In that case, a crystal is rapidly cooled at about -173 **, and the low temperature thermometry which collects diffraction data in the state has been spreading recently. in addition -- a crystal does not collapse when cooling -- a system -- it is necessary to devise solvent composition so that the whole may become vitrified
(3) In addition to diffraction data, topology is needed in order to conduct crystal structure analysis. Since the spacial configuration of EB-AP of the protein of a relative is strange, a phase problem must be solved by a heavy atom isomorphous replacement method. A heavy atom isomorphous

replacement method is a method of introducing a metal atom with big atomic numbers, such as mercury and platinum, into a crystal, and acquiring topology using contribution of X diffraction DETAHE of the big X-ray scattering ability of a metal atom. If the spacial configuration of wild type EB-AP is determined, the crystal structure of analogs, such as an enzyme variant and a reaction intermediate analog, can be determined with the molecular replacement method which used it. A molecular replacement method is the technique of performing structure determination in the protein which wants to determine a crystal structure using the spacial configuration, when the spacial configuration of the protein of a relative is known. For example, if the spacial configuration of the wild type of a certain protein is known, a molecular replacement method is applicable to the crystal structure determination of the variant protein or the protein by which chemical modification was carried out. About G74 D/I153T variant EB-AP, a crystal structure is determined for a molecular mechanism break through of the improvement in nucleoside compatibility by two amino acid substitution. About a reaction intermediate analog, in order to build a bond form model with a nucleoside, a crystal structure is determined. A nucleoside is changed into nucleoside 5'-phosphoric acid after the phosphate group supplied combines with EB-AP in the state where the covalent bond was carried out, i.e., reaction intermediate. Since the reaction intermediate of EB-AP was unstable, the structure could not be caught, but since it was not hydrolyzed instead of phosphoric acid when molybdic acid was the reaction intermediate analog which carried out the covalent bond, I thought that structure determination was possible. Details were shown in Examples 4, 6, and 7.

[0011](4) On computer graphics (CG), based on the molybdic acid connecting position in the three-dimensional structure of a reaction intermediate analog, make a nucleoside fit the hollow of the neighborhood and build a bond form model (drawing 3). A program like QUANTA of MSI (United States) and INSIGHT II is used for construction of a model, for example. Drawing 3 is a photograph which shows the crystal structure of the above-mentioned bond form model. Details were shown in Examples 5 and 8.

(5) Observe coupled models well and design the variation which increases compatibility with a nucleoside. In order to raise compatibility, a means to enhance a canal interaction, an electrostatic interaction, a hydrogen bond, a pi-pi interaction (interaction of the magnetic fields which the ring current of an aromatic ring generates), and a CH/pi interaction (interaction of the magnetic field which the ring current of an aromatic ring and the electron of a methyl group generate) can be considered. Since it is predicted that Ser72 interacts with the base of a nucleoside most strongly, It seems that the substitution to Phe, Tyr, and Trp enhances a hydrophobic interaction and a pi1pi interaction, the substitution to Val, Ile, and Leu enhances a hydrophobic interaction and a CH/pi interaction, and the substitution to Glu and Asp reinforces an electrostatic interaction and a hydrogen bond. A hydrophobic interaction etc. may be enhanced by the substitution to other amino acid, especially the substitution to the amino acid which has a more nearly long-chain side chain. It is expected that a pi1pi interaction will be formed between the aromatic ring of amino acid residue and the base of a nucleoside which were replaced also by replacing Leu16, Ser71, Ser73, and Glu104 by Phe, Tyr, and Trp. Formation of a hydrogen bond with the ribose of a nucleoside is expected by replacing Ile103 and Thr153 by more nearly long-chain hydrophilic residue. Since an opening will produce inside protein if it replaces by amino acid residue with small side chains [151 / which was located near the nucleoside binding site and has been buried in the inside of protein / Thr], such as Ser, Ala, and Gly, The pliability of a nucleoside binding site is expected whether to be able to take increase and the conformation which was suitable by combination with a nucleoside. Although Leu140 has separated not less than 10A from Ser72, it is located in the nearest to a phosphate bond part in the spacial configuration of a reaction intermediate analog. Therefore, when replacing this residue, the structure around a phosphate bond part in reaction intermediate changed, and it was thought by extension that influence also attained to the structure of a nucleoside binding site and fluctuation. If it replaces without Lys which has bulkier Phe and positive charge for this residue, and Glu which has a negative charge, it is hoped that compatibility with a nucleoside may change. The above-mentioned variation is introduced to G74 D/I153T variant EB-AP at the beginning. However, the enzyme variant made into the object of introduction is not restricted to G74 D/I153T variant EB-AP. For example, it is also possible to introduce variation into 10 residue substitution variant EB-AP. Variation is carried out to introducing to G74 D/I153T variant EB-AP. In this case, the enzyme variant produced turns

into 3 residue substitution enzyme variant. Details were shown in Example 9.
(6) Produce the plasmid which contains the gene which encodes variant EB-AP by the PCR method. A plasmid is introduced into Escherichia coli (Escherichia coli) JM109, and variant EB-AP is made to produce. Km value which becomes an index of compatibility to the inosine of variant EB-AP, and the phosphoryl-group-transfer activity which changes inosine into 5'-inosinic acid are measured, and the performance of variant EB-AP is evaluated. It is thought that it depends for the quantity of production of nucleoside 5'-phosphoric acid on Km value much. Pyrophoric acid reacts to EB-AP, phosphoric acid ion breaks away, and if a water molecule attacks this after the reaction intermediate of the form in which the phosphate group carried out the covalent bond to EB-AP is formed, a phosphate group will separate (phosphatase reaction). It means that pyrophoric acid was consumed vainly, without generating nucleoside 5'-phosphoric acid. On the other hand, if a nucleoside attacks reaction intermediate, a phosphate group will form a nucleoside and phospho monoester binding, and the generated nucleoside 5'-phosphoric acid will secede from EB-AP (phosphoryl-transfer reaction). It means that pyrophoric acid was utilized for nucleoside 5'-phosphoric acid generation. That is, if water and a nucleoside scramble for reaction intermediate, water wins and a nucleoside will win in phosphatase activity, phosphoryl-group-transfer activity will be demonstrated. If the compatibility over EB-AP of a nucleoside goes up (i.e., if Km value falls), a possibility that a phosphoryl-transfer reaction will be performed will become high. If the hydrophobicity near a phosphate bond part is improved and water becomes difficult to approach, phosphatase activity will become weaker and phosphoryl-group-transfer activity will become strong relatively. Details were shown in Example 10.
(7) Conduct the experiment which produces 5'-inosinic acid from inosine using Escherichia coli JM109 which introduced the plasmid containing the variant EB-AP gene in which Km value fell to and phosphoryl-group-transfer activity went up. A reaction is performed at 30 ** for 45 hours, and aging of 5'-inosinic acid quantity of production is monitored. Details were shown in Example 12.
(8) If the variation part in which Km is reduced is found out, by combining two or more residue, the compatibility over a nucleoside can improve further and the enzyme variant which became high [productivity] can be produced. Two or more mutation sites can be introduced by repeating site-specific mutation cumulatively. If the primer from which the portion of the base which encodes the amino acid residue which introduces variation in the case of introduction of site-specific mutation became a mix base is used, the library of a variant gene where specific amino acid residue was replaced by all the amino acid can be created. If the primer of a mix base is used for two or more parts and variation is introduced, the library of the variant gene which encodes the enzyme variant of various sorts dramatically can be created. Thus, the method of selecting the variant which introduced the library of the built gene into Escherichia coli and with which the amino acid substitution of high activity was combined from the library made to reveal is also effective.
[0012]If it is an enzyme with the space which the same active site and nucleoside as EB-AP can combine also except EB-AP, there is potential which can be used for production of nucleoside 5'-phosphoric acid. An active site has indispensable amino acid residue with activity, and must be arranged by the space position relation with suitable them. In EB-AP, Lys115, Arg122, His150, Arg183, and His189 are indispensable with activity, and it is possible to specify space position relations with the distance between Calpha of these 5 residue. In this invention, since three EB-AP crystal structures, a wild type, G74 D/I153T variant, and a reaction intermediate analog, were determined, Calpha interatomic distance of the activity residue in each structure was measured, and Table 1 was created. Since each distance distribution of Table 1 had the width which is about 1A, when beyond a distance (Table 1, a minimum) shorter 1A than the shortest distance was below a distance (Table 1, a maximum) longer 1A than longest distance, I thought that the active site called for could be formed. The physical relationship of five residue was shown in drawing 1 with the distance between Calpha of the maximum and the minimum. In MM-AP which is a relative enzyme of EB-AP, it was shown in Example 15 that it was checked that all the interatomic distances between activity residue have fitted in the range specified from the spacial configuration of EB-AP. By this example, although the example of G72 D/I151T variant instead of a wild type was shown, it is thought that there is no big difference to the spacial configuration of an active site at the same wild type and variant of an enzyme. This guess is supported by that the structure of the active site of the wild type of EB-AP and G74 D/I153T variant is fundamentally the same (refer to Table 1). In order to

phosphorylate a nucleoside and to change into nucleoside 5'-phosphoric acid, just the active site that comprises the 5 above-mentioned residue is insufficient, and a nucleoside must be able to combine with a suitable position in it. In EB-AP, a grooved space suitable for a nucleoside joining near the binding site of a phosphate group together exists in the molecule surface. [(drawing 3): It can display on computer graphics (CG) using drawing 10 in which the atomic coordinates of which attachment was done are shown - drawing 45.]This slot is specified as a space surrounded by 4 residue of Leu16, Ser72, Glu104, and His189. Even if it has an active site, the enzyme without the suitable space which a nucleoside combines is unsuitable as a nucleoside 5'-phosphoric acid production enzyme.
[0013]
[Table 1]

		野生型	G74D/I153T 変異型	反応中間体 アナログ	下限	上限
Lys115	Arg122	11.6Å	11.6Å	11.4Å	10.4Å	12.6Å
	His150	12.4Å	12.3Å	12.8Å	11.3Å	13.8Å
	Arg183	16.4Å	16.3Å	15.5Å	14.5Å	17.4Å
	His189	12.6Å	12.1Å	11.7Å	10.7Å	13.6Å
Arg122	His150	13.2Å	13.6Å	14.2Å	12.2Å	15.2Å
	Arg183	10.4Å	10.5Å	10.8Å	9.4Å	11.8Å
	His189	5.6Å	5.5Å	5.7Å	4.5Å	6.7Å
His150	Arg183	8.4Å	8.8Å	7.7Å	6.7Å	9.8Å
	His189	9.8Å	10.0Å	10.0Å	8.8Å	11.0Å
Arg183	His189	5.5Å	6.8Å	5.7Å	4.5Å	6.8Å

[0014]This invention provides preferably the substitution to other amino acid of Ser72, and Phe, Tyr, Trp, Val, Leu, Glu, Asp, Gln, Met, Thr, Arg and variant EB-AP that performed substitution to any one amino acid of Lys. The residue (residue number: 16, 70-71,73-76 and 100, 102-104, 106-108, 115, 148-154, 183) in Ser72 to 10A has a dramatically high possibility of interacting with a nucleoside, Variant EB-AP which performed substitution to other amino acid of these amino acid residue is provided. It includes here not only when amino acid being artificially replaced by substitution, but selecting other acid phosphatase belonging to the same enzymatic family as EB-AP which substitution produced in the nature. However, variant EB-AP containing mutational sites other than the above-mentioned amino acid residue can also provide this invention.
[0015]Also in other acid phosphatase belonging to the same enzymatic family as EB-AP, if amino acid variation of homologous is given, it is possible to use for manufacture of nucleoside 5'-phosphoric acid. However, the amino acid residue of EB-AP is not necessarily equivalent to the amino acid residue of the same number in other acid phosphatase. For example, Ser72 of EB-AP corresponds to Ala70 in MM-AP. Matching of the amino acid residue of two different protein, If the homology of both amino acid sequence is not less than about 20% and it is alignment (Sequence Alignment) of amino acid sequences, and about 20% or less, it will become clear by alignment (Threading) of the three-dimensional structure and an amino acid sequence. The former can perform the latters, such as BLAST, by programs, such as INSIGHT II. Amino acid sequence alignment of EB-AP and Enterobacter aerogenes (Enterobacter aerogenes) origin acidity HOFATAZE (EA-AP) using BLAST was shown in Example 14. BLAST should just obtain the file in accordance with the computer used among the files which exist in /blast/executable from ncbi.nlm.nih.gov using FTP. Details are described by http://genome.nhgri.nih.gov/blastall/blast#install about operation information.
[0016]Although attained by the improvement in compatibility with a nucleoside in many cases, it is attained by the shift of optimal pH, thermal stability improvement, etc. besides it, and deals in the improvement in nucleoside 5'-phosphoric acid productivity. The shift of optimal pH can be attained by changing pK of activity residue. [Protein engineering (Protein Engng.), the 1st volume, the 383-388th page (1998)] .Substitution to the glycine residue of the residue in which thermal stability improvement takes introduction of proline residue, and a counterclockwise twining helical structure [Protein

engineering, the 6th volume, the 85-91st page (1993)] The opening inside protein is filled. [Biochemistry (Biochemistry), the 32nd volume, the 6171-6178th page (1993)] ******** achievement is possible. It is effective in order to produce the variant whose nucleoside 5'-phosphoric acid productivity the compatibility of this spacial configuration of the nucleoside improved and improved, as explained to details above, but. This spacial configuration is effective not only in the compatibility over the nucleoside of an enzyme but changing compatibility with a phosphate donor. This enzyme can use various phosphoric ester compounds, such as polyphosphoric acid (salt), phenylphosphoric acid (salt), acetyl phosphate (salt), and carbamyl phosphate (salt), as a phosphate donor as indicated to JP,9-37785,A, but. It is possible to extend the substrate specificity of a phosphate donor or to raise the capacity factor of phosphoric acid by designing the variation which increases compatibility with a phosphoric ester compound in a way similar with having designed the variation which increases compatibility with a nucleoside.

[0017]

[Example]Hereafter, although an example explains this invention still more concretely, this invention is not limited to these examples.

[0018]Example 1 It crystallized using steamy diffusion with the crystallization hanging drop method of wild type EB-AP. 20mM buffer solution (pH 8.0) of sodium phosphate containing wild type EB-AP (concentration of 10mg/ml), the 100mM buffer solution of the trischloride containing the polyethylene glycol 400 of 45 (w/v %) -- an equivalent amount --- every (each 7-10microl). the well which filled 100mM buffer solution 500mul of the trischloride which carries out dropping mixing on the cover glass [SHIRIKONAIZEISHON / cover glass], and contains the polyethylene glycol 400 of 45 (w/v %) -- it covered so that a mixed liquor drop might fish and fall on (well), and it settled at 20 **. The crystal deposited 2 or 3 days afterward, and, two weeks afterward, it grew up to be a crystal of the shape of a hexagonal prism of a measurable size (about 0.3x0.3x1.2 mm) from one week. On the occasion of X-ray-data measurement, the crystal was moved to the 100mM buffer solution (pH 8.0) of the trischloride containing the polyethylene glycol 400 of 50 (w/v %). This crystal needed to care about the following point on handling. 1) Since a crystal collapsed very easily by contacting a container and tools from a drop (droplet) when taking out a crystal, the crystallization gestalt of the sitting dropping method could not be used (a crystal grows), but used the hanging drop method described here. 2) At ordinary temperature measurement, since a crystal deteriorated during measurement and resolution fell gradually, it needed to be measured under the low temperature service. Time until it mounts a crystal on a stage was shortened as much as possible, and it devised so that it might not expose to air. Using X-ray diffractometer R-AXIS IIc of Rigaku, X diffraction data was collected and the crystallographic parameter was determined. The space group was set to $P6_322$ and the grating constant became a=b=124.4A and c= 97.7 A. If it assumes that one subunit of the molecular weight 25000 is included in an unsymmetrical unit, the moisture content of a crystal will be 72%.

[0019]It crystallized using steamy diffusion with the crystallization hanging drop method of example 2 G74 D/I153T variant EB-AP. 20mM buffer solution (pH 8.0) of the trischloride containing G74 D/I153T enzyme variant (concentration of 20mg/ml), 20mM buffer solution of the trischloride containing the polyethylene glycol 400 of 38 (w/v %) -- an equivalent amount -- every (each 5microl). Dropping mixing was carried out on the cover glass [SHIRIKONAIZEISHON / cover glass], it covered so that a mixed liquor drop might fish and fall on the well which filled 20mM buffer solution 500mul of the trischloride containing the polyethylene glycol 400 of 38 (w/v %), and it settled at 20 **. The crystal deposited 2 or 3 days afterward, and, one week afterward, it grew up to be a plate crystal of a measurable size (about 0.7x0.4x0.2 mm). On the occasion of X-ray-data measurement, the crystal was moved to the 100mM buffer solution (pH 8.0) of the trischloride containing the polyethylene glycol 400 of 50 (w/v %). Using X-ray diffractometer R-AXIS IIc of Rigaku, X diffraction data was collected and the crystallographic parameter was determined. The space group became $P2_12_12_1$, and the grating constant became a= 138.0 A, b= 168.3 A, and c= 58.2 A. If it assumes that one hexamer molecule of the molecular weight 150000 is included in an unsymmetrical unit, the moisture content of a crystal will be 64%.

[0020]Example 3 It crystallized using the cocrystal-ized method for having used steamy diffusion by the crystallization sitting dropping method of the complex (reaction intermediate analog) of wild type

EB-AP and molybdic acid. 20mM buffer solution (pH 8.0) of sodium phosphate containing wild type EB-AP (concentration of 10mg/ml), the 100mM buffer solution of the trischloride containing the polyethylene glycol 400 of 40 (w/v %), and sodium molybdate of 1mM -- an equivalent amount -- every (each 15microl). Dropping mixing was carried out and it settled on the hollow of the bridge installed in the well which filled 500micro of 100mM buffer solution (pH 8.0) l of the trischloride containing the polyethylene glycol 400 of 40 (w/v %) at 20 **. The crystal deposited two to three days afterward, and, two weeks afterward, it grew up to be a crystal of the shape of a diamond-shaped rice cake of a measurable size (about 0.3x0.3x0.3 mm) from one week. On the occasion of X-ray-data measurement, the crystal was moved to the 100mM buffer solution (pH 8.0) of the trischloride containing the polyethylene glycol 400 of 50 (w/v %). Using X-ray diffractometer R-AXIS IIc of Rigaku, X diffraction data was collected and the crystallographic parameter was determined. The space group was set to $P3_121$ and the grating constant became a=b=86.6A and c= 205.3 A. If it assumes that three subunits of the molecular weight 25000 are included in an unsymmetrical unit, the moisture content of a crystal will be 58%.

[0021]Example 4 The X diffraction data to a maximum of 1.9A of crystal-structure-analysis resolution data of wild type EB-AP was measured. In ordinary temperature, since the damage by the exposure of X-rays was intense, the crystal measured by cooling quickly at -173 **. The Shigehara child derivative was screened by dipping a crystal into the solution of heavy metal salt. The diffraction data of the Shigehara child derivative crystal was obtained using Rigaku R-AXIS IIc. K_2PtCl_4 found out giving the good Shigehara child same type crystal from the difference Fourier figure with native data. By using the program RSPS, the coordinates of the only platina binding site of K_2PtCl_4 were searched for. It asked for the phase which carries out elaboration of these coordinates by the program MLPHARE, and is calculated. It asked for five mercury binding sites of the 2nd Shigehara child derivative $KHgI_4$-KI using this phase. After carrying out elaboration of the Shigehara child parameter of both K_2PtCl_4 and $KHgI_4$-KI simultaneously using MLPHARE, using program DM, solvent smoothing and histogram matching were performed and the phase was improved. Incidentally, anomalous dispersion data was also used about K_2PtCl_4. The electron density map calculated using this good phase was dramatically clear, and was able to fit almost all amino acid residue finely. The first model was built using the program QUANTA on the electron density map created with 2.8A resolution, and performed structure refinement using program X-PLOR. Electron density was not observed and 6 residue in the end of N, the 135 to 136th residue, and 1 residue in the end of C were not able to determine structure uniquely. The final model (drawing 4 - drawing 6) by which elaboration was carried out with 1.9A resolution contains all the 222 in 231 residue residue, 236 water molecules, and one molecule of sulfate ion. Sulfate ion originates in the ammonium sulfate used in refining processes. It is considered a match by the phosphate bond part of an active center.

The crystallographic reliability factor (R factor) using reflection of 8-1.9A resolution became 21.5%. The average temperature factor became $26A^2$ about the protein atom, and became $45A^2$ about the water molecule. When Ramachandran plot was created using the program PROCHECK, it was shown in the field where 93% of residue other than a glycine is the most preferred that 7% is located in a field desirable next. One subunit is contained in an unsymmetrical unit and a hexamer is formed of crystallographic symmetry. Atomic coordinates were shown in drawing 10 - drawing 45.

[0022]Drawing 4 is a CG photograph which shows the crystal structure of the hexamer molecule of EB-AP. The flow of alpha carbon atom was displayed with the ribbon model. The sulfate ion which marks an active center was displayed with the ball model. Drawing 5 is a CG photograph which shows the crystal structure of the subunit of EB-AP. The flow of alpha carbon atom was displayed with the ribbon model. The sulfate ion which marks an active center was displayed with the ball model. Drawing 6 is a figure showing the active site architecture of EB-AP. Sulfate ion was shown in the center. The dotted line showed the hydrogen bond. Drawing 10 is a figure showing the crystallography data (1) of the structure of EB-AP. Drawing 11 is a figure showing the crystallography data (2) of the structure of EB-AP. Drawing 12 is a figure showing the crystallography data (3) of the structure of EB-AP. Drawing 13 is a figure showing the crystallography data (4) of the structure of EB-AP. Drawing 14 is a

figure showing the crystallography data (5) of the structure of EB-AP. Drawing 15 is a figure showing the crystallography data (6) of the structure of EB-AP. Drawing 16 is a figure showing the crystallography data (7) of the structure of EB-AP. Drawing 17 is a figure showing the crystallography data (8) of the structure of EB-AP. Drawing 18 is a figure showing the crystallography data (9) of the structure of EB-AP. Drawing 19 is a figure showing the crystallography data (10) of the structure of EB-AP. Drawing 20 is a figure showing the crystallography data (11) of the structure of EB-AP. Drawing 21 is a figure showing the crystallography data (12) of the structure of EB-AP. Drawing 22 is a figure showing the crystallography data (13) of the structure of EB-AP. Drawing 23 is a figure showing the crystallography data (14) of the structure of EB-AP. Drawing 24 is a figure showing the crystallography data (15) of the structure of EB-AP. Drawing 25 is a figure showing the crystallography data (16) of the structure of EB-AP. Drawing 26 is a figure showing the crystallography data (17) of the structure of EB-AP. Drawing 27 is a figure showing the crystallography data (18) of the structure of EB-AP. Drawing 28 is a figure showing the crystallography data (19) of the structure of EB-AP. Drawing 29 is a figure showing the crystallography data (20) of the structure of EB-AP. Drawing 30 is a figure showing the crystallography data (21) of the structure of EB-AP. Drawing 31 is a figure showing the crystallography data (22) of the structure of EB-AP. Drawing 32 is a figure showing the crystallography data (23) of the structure of EB-AP. Drawing 33 is a figure showing the crystallography data (24) of the structure of EB-AP. Drawing 34 is a figure showing the crystallography data (25) of the structure of EB-AP. Drawing 35 is a figure showing the crystallography data (26) of the structure of EB-AP. Drawing 36 is a figure showing the crystallography data (27) of the structure of EB-AP. Drawing 37 is a figure showing the crystallography data (28) of the structure of EB-AP. Drawing 38 is a figure showing the crystallography data (29) of the structure of EB-AP. Drawing 39 is a figure showing the crystallography data (30) of the structure of EB-AP. Drawing 40 is a figure showing the crystallography data (31) of the structure of EB-AP. Drawing 41 is a figure showing the crystallography data (32) of the structure of EB-AP. Drawing 42 is a figure showing the crystallography data (33) of the structure of EB-AP. Drawing 43 is a figure showing the crystallography data (34) of the structure of EB-AP. Drawing 44 is a figure showing the crystallography data (35) of the structure of EB-AP. Drawing 45 is a figure showing the crystallography data (36) of the structure of EB-AP.

[0023]Example 5 Since it exceeds 100mM, Km value of compatibility to wild type EB-AP and EB-AP of the guess inosine of the bond form model of 5'-inosinic acid is not so high as it can determine a bond form by X ray crystal structure analysis. After actually soaking the compound used as inhibitor of EB-AP, such as glucose 6-sulfate and adenosine thio monophosphate, into the crystal of wild type EB-AP, collected X diffraction data, and created the electron density map, but. The electron density corresponding to these compounds was not observed. Then, we decided to guess the bond form of 5'-inosinic acid and EB-AP (what is called a docking study) using computer graphics. The program used QUANTA. Among the crystal structure, since sulfate ion was found out in the center of an active site, the phosphate group of 5'-inosinic acid was piled up here. Since it was known that G74D and the variation of I153T will reduce Km value to EB-AP of 5'-inosinic acid, it judged that 5'-inosinic acid was combined with the place in which a long distance is not from G74 and I153, and the position of 5'-inosinic acid was decided. The atom which constitutes 5'-inosinic acid, and the atom which constitutes EB-AP were kept from colliding mutually in that case. In this way, in the built model, if I153 is set to T, the gamma acid matter atom of the side chain of threonine and 2' hydroxyl group of the ribose of inosine which were replaced will form a hydrogen bond. When an electrostatic potential indication of EB-AP is given using the program GRASP, the inosine base which is tinged with positive charge is interacting with the field which is tinged with the negative charge on the EB-AP molecule surface.

It was suggested that a model will seemingly be reasonable.

[0024]The ratio of phosphoryl-group-transfer activity [as opposed to phosphatase activity in crystal-structure-analysis G74 D/I153T variant EB-AP of example 6 G74 D/I153T variant EB-AP] is increasing.

In connection with it, the production capacity of nucleoside 5'-phosphoric acid is also improving. It is considered as a cause that it improved that this Km value with a nucleoside fell, i.e., compatibility with a nucleoside. When determining the crystal structure of this variant EB-AP and comparing with the crystal structure of wild type EB-AP, it was expected that the molecular mechanism of the improvement in compatibility with a nucleoside would be clarified. At ordinary temperature, the X diffraction data to a maximum of 2.4A resolution data was measured. It was expected that estimate from the molecular weight of the volume of a unit cell, a space group, and an enzyme, and one molecule of a hexamer is contained in an unsymmetrical unit. Then, it analyzed with the molecular replacement method using the program amore by making hexamer structure of wild type EB-AP into a search model. In rotation search, the data of 10-4A resolution was used for the data of 10-3A resolution in translation search. The correct answer of both searches appeared as top peaks. When elaboration was performed by using a molecule as a rigid body, the R factor fell to 37.3%. Then, it carried out by having repeated the structure correction on the graphics using QUANTA, and the structure refinement using X-PLOR, and the model of 19.9% of the R factor was obtained in 10-2.4A resolution. When the coupled models of 5'-inosinic acid and G74 D/I153T variant EB-AP were created by the same method as Example 5, it was expected that the gamma acid matter atom of the side chain of replaced Thr153 forms the hydroxyl group and hydrogen bond of a ribose of inosine. By comparing a temperature factor showed that the direction of G74 D/I153T variant EB-AP was large compared with a wild type in fluctuation of the loop containing Asp74 to which one more substitution was performed. Although it is expected that this loop interacts with the base of inosine, when fluctuation became large, a possibility of becoming easy to carry out combination with a base is suggested.

[0025]Example 7 In the enzyme reaction of crystal-structure-analysis EB-AP of the complex (reaction intermediate analog) of wild type EB-AP and molybdic acid, first, monoester phosphate combination is cut and a phosphate group forms His189 of activity residue, and a covalent bond. The enzyme molecule of this state is called reaction intermediate. Promptly, reaction intermediate receives the attack by water or alcohol, and, as a result, phosphoric acid ion secedes from it. If water attacks, phosphatase activity will be demonstrated, and if alcohol attacks, phosphoryl-group-transfer activity will be demonstrated. Anyway, reaction intermediate is unstable and it is impossible to determine the structure by X ray crystal structure analysis. However, since that (reaction intermediate analog), as for, molybdic acid carried out the covalent bond to His189 instead of phosphoric acid does not receive the attack by water, it exists in stability. In a phosphoryl-transfer reaction, a phosphate acceptor combines with reaction intermediate and monoester phosphate combination is formed. Therefore, it is more suitable to use reaction intermediate structure rather than isolation mold structure in the purpose of presuming a bond form with a nucleoside. Crystal structure analysis of the reaction intermediate analog was conducted in order to perform a docking study with reaction intermediate and a nucleoside. At ordinary temperature, the X diffraction data to a maximum of 2.4A resolution data was measured. It was expected that estimate from the molecular weight of the volume of a unit cell, a space group, and an enzyme, and three half of a hexamer, i.e., subunits, is included in an unsymmetrical unit. Then, with the threefold rotation axis, the trimer structure where each other was connected was created and it was considered as the search model of the molecular replacement method. In rotation search, the data of 10-4A resolution was used for the data of 10-3A resolution in translationsearch. The correct answer of both searches appeared as top peaks. When elaboration was performed by using a molecule as a rigid body, the R factor fell to 42.4%. Then, it carried out by having repeated the structure correction on the graphics using QUANTA, and the structure refinement using X-PLOR, and the model of 22.3% of the R factor was obtained in 8-2.4A resolution. Three molecule half of a hexamer, i.e., subunits, is included in an unsymmetrical unit.

[0026]The bond form model was built using QUANTA on the guess computer graphics of the bond form model of example 8 EB-AP reaction intermediate and inosine (drawing 3). Molybdic acid was transposed to phosphoric acid as it was. Inosine was placed near the nucleoside portion of 5'-inosinic acid in the bond form model of wild type EB-AP and 5'-inosinic acid. However, although it is natural, since inosine does not have monoester phosphate combination, flexibility is higher [inosine] than making 5'-inosinic acid dock. Therefore, inosine tuned the position of inosine finely and considered it

as the bond form model so that it might combine with the molecule surface of EB-AP in the more desirable state. We decided to use this model for the design of subsequent enzyme variants.
[0027]Example 9 According to the model built in design example 8 of variant EB-AP which aimed at the improvement in compatibility with a nucleoside, a possibility that the side chain of Ser72 would interact with the base of inosine was suggested. When this residue was replaced by aromatic amino acid, such as phenylalanine, tyrosine, and tryptophan, the pi-pi interaction arose between the aromatic ring and the nucleoside base, and it was predicted that the compatibility over EB-AP of a nucleoside improves. Similarly, if it replaces by branched chain hydrophobic amino acid, such as valine, leucine, and isoleucine, When replaced by the amino acid which the CH/pi interaction arose between the branched chain hydrophobic group and the nucleoside base, and is tinged with negative charges, such as glutamic acid and aspartic acid, it paid well to the positive charge of a nucleoside base, and electrostatic, and improvement in compatibility was expected. Then, in order that phosphoryl-group-transfer activity may improve further the phosphoryl-group-transfer activity of relatively increasing G74 D/I153T variant EB-AP rather than phosphatase activity, We decided to produce S72F of this variant EB-AP, S72Y, S72W, S72V, S72E, and an S72D variant. We decided to also produce the variant which replaced S72 by other amino acid. Incidentally, these variants serve as 3 residue substitution variant EB-AP.
[0028]In order to build nine kinds of variant EB-AP for revealing Example 10Ser72 by production Escherichia coli JM109 of 3 residue substitution variant EB-AP replaced to other amino acid, Plasmid pEPI340 containing a G74 D/I153T variant EB-AP gene was used as a mold of the site-directed-mutagenesis method using PCR. pEPI340 is the plasmid which added the variation of G74 D/I 153T to plasmid pEPI305. A deer is carried out and the base sequence of these plasmids pEPI305 and pEPI340 is specified in Table 12 of the paragraph number (0143) of JP,10-201481,A. It is named AJ13144 and the international deposit of the stock which made plasmid pEPI305 hold to Escherichia coli JM109 is carried out to National Institute of Bioscience and Human-Technology, the Ministry of International Trade and Industry, as FERM BP-5423. [Refer to the paragraph number (0105) of the above-mentioned publication before examination - (0110) a statement.] .Variation uses "a quick change site-directed-mutagenesis kit (Quickchange Site-Directed Mutagenesis Kit)" of Stratagene (Stratagene) (United States), and according to the protocol of the manufacturer, It introduced using the primer (drawing 7, array numbers 11-61 of an array table) corresponding to various enzyme variants. Escherichia coli XL-1 was transformed using the output of an PCR reaction. The transformed cell was smeared on L agar-medium plate containing 100 microl./ml ampicillin, and it incubated at 37 ** for 16 hours. The generated colony was extracted, and it cultivated, shaking overnight by L culture medium containing 100 microl./ml ampicillin. FlexiPrep Kit of Pharmacia Corp. (Sweden) was used after collecting biomasses by centrifugal separation from culture medium, and the plasmid was extracted according to the protocol of the manufacturer. The base sequence which encodes 3 residue substitution variant EB-AP in some numbers was checked by DNA sequence analysis. Composition of the primer set shown in drawing 7 was entrusted to Japanese Bio-Service.
[0029]Example 11 Escherichia coli JM109 which introduced the plasmid containing the phosphoryl-group-transfer activity of variant EB-AP and the measurement various 3 residue substitution variant EB-AP genes of a velocity constant was inoculated into 50 ml of L culture media containing 100 microl./ml ampicillin, and it cultivated at 37 ** for 16 hours. Biomasses were collected by centrifugal separation from culture medium, and it was suspended to 3 ml of 25mM phosphoric acid buffers (pH 7.0), and crushed by performing ultrasonication for 20 minutes at 4 **. The treating solution was centrifuged and the cell-free extract was prepared except for the insoluble fraction. It checked by SDS-PAGE that each EB-AP3 residue substitution enzyme variant was revealed. The expression amount was about 20% of quality of total protein. The phosphoryl-group-transfer activity of the cell-free extract was measured on condition of the following. 2mM inosine, 100mM sodium pyrophosphate, a 100mM acetic acid buffer (pH 4.0), and the reaction mixture (1 ml) containing the cell-free extract of 100microl were incubated for 10 minutes at pH 4 and 30 **. After adding 1N chloride 200mul and stopping a reaction, under [a fixed quantity / inosinic acid / which was generated except for precipitate by centrifugal separation / 5'-]. The relative activity set to 1 showed 5'-inosinic acid generated amount when G74 D/I153T variant EB-AP which set phosphoryl-group-transfer activity of 3 residue substitution variant EB-AP as the object which introduces variation in some numbers was

used. Then, Km value to the inosine in the phosphoryl-transfer reaction of 3 residue substitution variant EB-AP was measured on condition of the following in some numbers. The reaction mixture (1 ml) containing 100mM sodium pyrophosphate, a 100mM acetic acid buffer (pH 4.0), 10-100mM inosine, and the cell-free extract of 100microl was incubated for 10 minutes at pH 4 and 30 **. After adding 1N chloride 200mul and stopping a reaction, under [a fixed quantity / inosinic acid / which was generated except for precipitate by centrifugal separation / 5'-]. Km value was computed by Hanes-Woolf plot. A result is shown in Table 2.

[0030]

[Table 2]

	Km値	リン酸転移活性
S72F/G74D/I153T	20mM	2.80
S72Y/G74D/I153T	25mM	2.04
S72W/G74D/I153T	30mM	1.71
S72D/G74D/I153T	33mM	1.59
S72V/G74D/I153T	40mM	2.46
S72E/G74D/I153T	40mM	3.19
S72M/G74D/I153T	46mM	1.94
S72T/G74D/I153T	50mM	1.91
S72L/G74D/I153T	57mM	2.24
S72R/G74D/I153T	59mM	1.99
S72Q/G74D/I153T	77mM	2.42
S72K/G74D/I153T	78mM	1.53
S72P/G74D/I153T	109mM	1.34
S72A/G74D/I153T	115mM	0.78
S72N/G74D/I153T	124mM	0.43
S72G/G74D/I153T	137mM	0.43
S72H/G74D/I153T	n.d.	n.d.
G74D/I153T	100mM	1.00
10残基置換変異型	40mM	1.44

[0031]Km value to the inosine of all the variants (S72F, S72Y, S72W, S72V, S72L, S72E, S72D) predicted that compatibility with inosine will improve by the pi-pi interaction, the CH/pi interaction, and an electrostatic interaction in Example 9, It fell compared with the thing of G74 D/I153T variant EB-AP which does not carry out variation introduction, and the compatibility over inosine improved. Improvement was found also about phosphoryl-group-transfer activity. The variant of the improvement which introduced S72F especially was remarkable in Km value, phosphoryl-group-transfer activity, and both sides. The aromatic ring and inosine base of phenylalanine carry out pi-pi interaction by suitable physical relationship, and it is surmised that improvement in compatibility was achieved. Km value of S72M, S72T,R [S72], S72Q, and S72K variant also fell. It is thought that some desirable interactions, such as a hydrophobic interaction and a hydrogen bond, arose between these amino acid residue and a nucleoside base. Incidentally, a gene was not able to be produced about S72I. Since there was a danger of making the mistaken SS linkage forming, S72C was not produced. High performance chromatography (HPLC) analyzed 5'-inosinic acid on condition of the following. column: -- Cosmosil 5C18-AR (4.6x150 mm) Nacalai Tesque, Inc. product mobile phase: -- 5mM potassium phosphate buffer (pH 2.8) / methanol =95/5 rate-of-flow: -- 1.0 ml/min temperature: -- room temperature detection: -- UV245nm [0032]Production G74 D/I153T variant of 5'-inosinic acid using Escherichia coli JM109 which introduced the example 12 S72 F/G74 D/I153T variant EB-AP gene, Escherichia coli JM109 which introduced the plasmid containing 10 residue substitution variant and an S72 F/G74 D/I153T variant EB-AP gene was inoculated into 50 ml of L culture media containing ampicillin 100microg/ml and IPTG 1mM, and it cultivated at 37 ** for 16 hours. 12 g/dl of pyrophoric acid and 6 g/dl of inosine are dissolved in an acetic acid buffer (pH 4.0), The reaction was performed at 30 ** for 24 hours, maintaining [added the biomass of Escherichia coli JM109 which

introduced each of above-mentioned variant EB-AP genes into this so that it might become 100 mg/dl by dry cell weight, and] pH to 4.0. The result of having measured the quantity of generated 5'-inosinic acid was shown in Table 3. As for the generated inosinic acid, the byproduction of 2'-inosinic acid and 3'-inosinic acid was not accepted at all only from 5'-inosinic acid. Although 7.5 g/dl of 5'-inosinic acid carried out generation accumulation at the reaction using Escherichia coli JM109 which introduced the plasmid containing a G74 D/I153T variant EB-AP gene, even if it developed reaction time, accumulation did not increase more than it. At the reaction using Escherichia coli JM109 which introduced the plasmid containing a 10 residue substitution variant EB-AP gene, accumulation improved and 5'-inosinic acid which is 12.1 g/dl carried out generation accumulation. It designed based on the spacial configuration, and at the reaction using Escherichia coli JM109 which introduced the plasmid containing the built S72 F/G74 D/I153T variant EB-AP gene, productivity improved further and 5'-inosinic acid which is 13.2 g/dl carried out generation accumulation.

[0033]

[Table 3]

導入した変異型酵素遺伝子	生成イノシン酸(g/dl)
G74D/I153T	7.5
10残基置換変異型	12.1
S72F/G74D/I153T	13.2

[0034]Measurement S72F variation of the phosphoryl-group-transfer activity of 3 residue substitution variant EB-AP and a velocity constant which introduced example 13 L16W, S71W, S73W, E104F, and E104W variation, Since it was thought that compatibility with inosine was raised by the pi-pi interaction, it searched for other amino acid residue which can plan a pi-pi interaction with an inosine base by substitution to aromatic ring amino acid on computer graphics. As a result, a possibility that the aromatic ring replaced by L16W, S71W, S73W, E104F, and E104W variation would interact with an inosine base was suggested. Then, a method which described these five sorts of 3 (G74 D/I153T variant EB-AP was used as base) residue substitution variant EB-AP in the Example 10 (the primer corresponding to each enzyme variant was shown in drawing 8.) It produced with the array numbers 62-76 of the array table, and phosphoryl-group-transfer activity and a velocity constant were measured by the method described in the Example 11. A result is shown in Table 4. Although phosphoryl-group-transfer activity fell also in which enzyme variant, Km value fell with all the enzyme variants, and it was suggested that the compatibility with inosine improved. Although Leu16 had separated 10A from Ser72 an interaction with inosine is predicted to be (in distance between Calpha), even if separated to this extent, it was shown that an interaction with inosine is possible. Composition of the primer set shown in drawing 8 was entrusted to Japanese Bio-Service.

[0035]

[Table 4]

	Km値	リン酸基転移活性
L16W/G74D/I153T	33mM	0.21
S71W/G74D/I153T	75mM	0.26
S73W/G74D/I153T	29mM	0.77
E104F/G74D/I153T	61mM	0.65
E104W/G74D/I153T	67mM	0.26
G74D/I153T	100mM	1.00
10残基置換変異型酵素	40mM	1.44

[0036]Example 14. In production EB-AP of 5'-inosinic acid using Escherichia coli JM109 which introduced production and this gene of the variant enzyme gene of Enterobacter aerogenes

(Enterobacter aerogenes) origin acid phosphatase (EA-AP). It was presupposed that a variation [homologous / variation / three / of S72 F/G74 D/I 153T which raised the phosphoryl-group-transfer activity of inosine] is introduced into EA-AP. The result of having aligned the amino acid sequence of EB-AP and EA-AP using the program BLAST is shown in drawing 9. It was shown that Ser72/Gly 74-/Ile153 of EB-AP corresponds to Ala70/Gly 72-/Ile151 in EA-AP. Then, A70 F/G72 D/I151T variant EA-AP was produced by the method described in the Example 10. It carried out using Escherichia coli JM109 which introduced the plasmid containing a variant enzyme gene by the method which described production of 5'-inosinic acid in the Example 12 from inosine. A result is shown in Table 5. A70 F/G72 D/I151T variant EA-AP showed 5'-inosinic acid productivity equivalent to S72 F/G74 D/I153T variant EB-AP.

[0037]

[Table 5]

導入した変異型酵素遺伝子	生成イノシン酸(g/dl)
EA-AP A72F/G74D/I153T	13.1
EB-AP S72F/G74D/I153T	13.2

[0038]Drawing 9 is a figure showing the result of having performed amino acid sequence alignment of EB-AP and Enterobacter aerogenes origin acid phosphatase (EA-AP) by the program BLAST. The upper row is EB-AP and the lower berth is EA-AP. If it is similar amino acid residue even if the residue name is not the same, if both amino acid residue is the same, + will be displayed on the middle. The position of the 72nd residue (Ser72) of EB-AP was marked by [72]. Residue corresponding by EA-AP is Ala70.

[0039]Example 15 G72 D/I151T double mutant of MM-AP was crystallized using crystallization of Morganella MORUGANI origin acid phosphatase (MM-AP) origin G72 D/I151T enzyme variant, and steamy diffusion with a crystal-structure-analysis hanging drop method. The protein solution (concentration of 40mg/ml) concerned, and the polyethylene glycol 1000 of 25 (w/v %), 25mM ammonium sulfate, 125mM citrate buffer solution containing 25mM DTT (pH 4.8) an equivalent amount -- every (each 5microl). Carry out dropping mixing on the cover glass [SHIRIKONAIZEISHON / cover glass], and The polyethylene glycol 1000 of 25 (w/v %), 25mM ammonium sulfate, 125mM citrate buffer solution containing 25mM DTT (pH 4.8) It covered so that a mixed liquor drop might fish and fall on the well which filled 500microl, and it settled at 20 **. The crystal deposited 2 or 3 days afterward, and, one week afterward, it grew up to be a measurable size (about 0.4x0.4x0.3 mm). Using X-ray diffractometer R-AXIS IIc of Rigaku, X diffraction data was collected and the crystallographic parameter was determined. The space group became $P2_12_12_1$ and the grating constant became a= 90.64 A, b= 119.74 A, and c= 136.14 A. The diffraction data to 2.6A resolution data was measured by 100K on Tsukuba and high energy research institute synchrotron radiation institution BL-6B. It was expected that estimate from the molecular weight of the volume of a unit cell, a space group, and an enzyme, and one molecule of a hexamer is contained in an unsymmetrical unit. Then, it analyzed with the molecular replacement method using the program amore by making hexamer structure of wild type EB-AP into a search model. In rotation search, the data of 10-4A resolution was used for the data of 10-3A resolution in translation search. The correct answer of both searches appeared as top peaks. After performing elaboration by using a molecule as a rigid body, it carried out by having repeated the structure correction on the graphics using QUANTA, and the structure refinement using X-PLOR, and the model of R factor 0.197 was obtained in 10-2.6A resolution. The distance between Calpha atoms of five activity residue (Lys113, Arg120, His148, Arg181, His187) shown in drawing 1 was shown in Table 6. In MM-AP which is a relative enzyme of EB-AP, it was checked that all the interatomic distances between activity residue have fitted in the range specified from the spacial configuration of EB-AP.

[0040]

[Table 6]

		G74D/I153T 変異型EB-AP	下限	上限
Lys113	Arg120	11.3Å	10.4Å	12.6Å
	His148	12.6Å	11.3Å	13.8Å
	Arg181	16.3Å	14.5Å	17.4Å
	His187	12.5Å	10.7Å	13.6Å
Arg120	His148	14.0Å	12.2Å	15.2Å
	Arg181	10.9Å	9.4Å	11.8Å
	His187	6.1Å	4.5Å	6.7Å
His148	Arg181	8.9Å	6.7Å	9.8Å
	His187	10.2Å	8.8Å	11.0Å
Arg181	His187	5.4Å	4.5Å	6.8Å

[0041]In production of 10 residue substitution variant EB-AP which introduced example 16 A72F and A72E variation, and the measurement example 11 of phosphate transfer activity and a velocity constant, S72F to which Km value was reduced most, and the S72E variation which improved activity most were introduced into 10 residue substitution variant EB-AP. In 10 residue substitution variant EB-AP, since Ser72 is replaced by Ala, A72F and A72E variation will be introduced actually. If based on wild type EB-AP, 10 residue is replaced for both sides. A method which described these two sorts of variants in the Example 10 (the primer corresponding to each enzyme variant was shown in drawing 46.) It produced with the array numbers 77-82 of the array table. As a mold of the site-directed-mutagenesis method using PCR, plasmid pEMP370 (JP,9-37785,A, Example 19) containing a 10 residue substitution variant EB-AP gene was used. Phosphate transfer activity and a velocity constant were measured by the method described in the Example 11. A result is shown in Table 7. The relative activity which set 5'-inosinic acid generated amount when G74 D/I153T variant EB-AP was used to 1 showed phosphate transfer activity. Also in which enzyme variant, Km value fell notably. About phosphate transfer activity, it went up by A72E variation to having fallen by A72F variation.

[0042]

[Table 7]

	Km値	リン酸転移活性
A72F/10残基置換変異型酵素	9mM	0.11
A72E/10残基置換変異型酵素	15mM	2.30
10残基置換変異型酵素	40mM	1.44

[0043]Production A72F / 10 residue substitution variant EB-AP of 5'-inosinic acid using Escherichia coli JM109 which introduced example 17 A72F / 10 residue substitution variant EB-AP, and A72E / 10 residue substitution variant gene, and A72E / 10 residue substitution variant gene. The production experiment of 5'-inosinic acid using Escherichia coli JM109 which introduced the included plasmid was conducted by the method described in the Example 12. A result is shown in Table 8. The accumulated dose of 5'-inosinic acid increased both variants.

[0044]

[Table 8]

導入した変異型酵素遺伝子	生成イノシン酸(g/dl)
A72F/10残基置換変異型	13.9
A72E/10残基置換変異型	13.9
10残基置換変異型	12.1

[0045]By measurement I103D variation of production of variant EB-AP which introduced example 18 I103D and T153N variation, phosphate transfer activity, and a velocity constant. It was suggested with the model of drawing 3 that replaced Asn forms the hydroxyl group and hydrogen bond of a ribose by that replaced Asp carries out an inosine base and an electrostatic interaction and T153N variation. Then, a method which introduced such residue into G74 D/I153T variant EB-AP, and described I103 D/G74 D/I153T variant EB-AP and G74 D/I153N variant EB-AP in the Example 10 (the primer corresponding to each enzyme variant was shown in drawing 47.) We decided to produce

with the array numbers 83-88 of an array table. Phosphate transfer activity and a velocity constant were measured by the method described in the Example 11. A result is shown in Table 9. The relative activity which set 5'-inosinic acid generated amount when G74 D/I153T variant EB-AP was used to 1 showed phosphate transfer activity. It was suggested that Km value fell and the compatibility of both variants with inosine improved although phosphoryl-group-transfer activity fell.

[0046]

[Table 9]

	Km値	リン酸転移活性
I103D/G74D/I153T	51mM	0.09
G74D/I153N	38mM	0.18
G74D/I153T	100mM	1.00

[0047]Although production of variant EB-AP which replaced Example 19Leu140 by Phe, Glu, and Lys, and measurement Leu140 of phosphate transfer activity and a velocity constant have separated not less than 10A from Ser72, they are located in the nearest to a phosphate bond part in the spacial configuration of a reaction intermediate analog. Therefore, when replacing this residue, the structure around a phosphate bond part in reaction intermediate changed, and it was thought by extension that influence also attained to the structure of a nucleoside binding site and fluctuation. If it replaces without Lys which has bulkier Phe and positive charge for this residue, and Glu which has a negative charge, it is hoped that compatibility with a nucleoside may change. Variation decided that phosphate transfer activity introduces into high A72E / 10 residue substitution product in Example 16. A method which described these three sorts of variants in the Example 10 (the primer corresponding to each enzyme variant was shown in drawing 46.) It produced with the array numbers 89-97 of the array table. As a mold of the site-directed-mutagenesis method using PCR, the plasmid containing A72E / 10 residue substitution variant EB-AP gene was used. Phosphate transfer activity and a velocity constant were measured by the method described in the Example 11. A result is shown in Table 10. The relative activity which set 5'-inosinic acid generated amount when G74 D/I153T variant EB-AP was used to 1 showed phosphate transfer activity.

[0048]

[Table 10]

	Km値	リン酸転移活性
A72E/L140F/10残基置換変異型酵素	9mM	1.66
A72E/L140K/10残基置換変異型酵素	78mM	0.07
A72E/L140E/10残基置換変異型酵素	322mM	0.16
A72E/10残基置換変異型酵素	15mM	2.30

[0049]Km value fell [the variant which introduced L140F]. On the contrary, L140K and L140E variation raised Km substantially.

[0050]example 20 Enterobacter aerogenes . IFO. 12010 . Acid phosphatase of the Enterobacter aerogenes IFO12010 origin from the culture object of refining of origin wild type acid phosphatase and Escherichia coli JM109/pENP110 of example 24 statement of determination JP,10-201481,A of N-terminal-amino-acid arrangement. It refined, N-terminal-amino-acid arrangement was determined, and the amino acid sequence of maturation protein was determined. Escherichia coli JM109/pENP110 is the bacillus which introduced the acid phosphatase gene of the Enterobacter aerogenes IFO12010 origin into 109 shares of Escherichia coli JM, and produces this acid phosphatase. The amino acid sequence of the precursor protein expected from the base sequence of this acid phosphatase gene is arrangement shown in the array number 10 of an array table. 50 ml of nutrient media (pH 7.0) containing 1 g/dl of peptone, 0.5 g/dl of yeast extracts, and 1 g/dl of salt were put into a 500-ml Sakaguchi flask, and it heat-sterilized for 20 minutes at 120 **. One platinum loop of Escherichia coli JM109/pENP110 was inoculated into this, and shaking culture was carried out at 30 ** for 16 hours. The biomass which collected biomasses from culture medium by centrifugal separation was suspended to the 100mM potassium phosphate buffer (pH 7.0) of 100 ml, ultrasonication was performed for 20 minutes at 4 **, and the biomass was crushed. The treating solution was

centrifuged and the cell-free extract was prepared except for the insoluble fraction. Ammonium sulfate was added so that it might become this cell-free extract with saturation 30%. After removing the precipitate generated by centrifugal separation, additional addition of the ammonium sulfate was carried out so that it might become digestive liquor with saturation 60%. Centrifugal separation recovered the generated precipitate and it dissolved in the 100mM potassium phosphate buffer. After dialyzing this crude enzyme liquid 3 times to 500 ml of 100mM potassium phosphate buffers (pH 7.0), It charged in DEAE-TOYOPARU 650M column (phi3.0x10.0cm) equilibrated with 20mM potassium phosphate buffer (pH 7.0), and 20mM potassium phosphate buffer (pH 7.0) washed. Since phosphate transfer activity suited the bypassing fraction, the fractions concerned were collected. Ammonium sulfate was added so that it might become this activity fraction with saturation 35%, and this was made to stick to the butyl-Toyopearl column (phi3.0x7.0cm) equilibrated with 20mM potassium phosphate buffer (pH 7.0) which contains saturated ammonium sulfate 35%. This was eluted by the linear concentration gradient of the saturated potassium phosphate buffer (pH 7.0) 20% from saturation 35%. CM-TOYOPARU equilibrated with 10mM potassium phosphate buffer (pH 6.0) after collecting activity fractions and dialyzing to 10mM potassium phosphate buffer (pH 6.0) 1L. It was made to stick to a column (phi3.0x7.0cm). This was eluted by the linear concentration gradient of the potassium phosphate buffer (pH 6.0) containing 300mM potassium chloride from 0mM. These activity fractions were collected. The above operation refined the enzyme in which phosphate transfer activity is shown about 5 times with the recovery rate of about 16% more nearly eventually than a cell-free extract. This enzyme preparation was uniform in SDS-polyacrylamide electrophoresis. It is DITC about these refining enzymes. Membrane It is made to stick to [a milli gene / bio-search (Milligen/Biosearch) company make], When the amino acid sequence of the amino terminal was determined using Prosequencer 6625 (a milli gene / bio-search company make), the amino acid sequence of the amino terminal of 5 residue shown in the array number 98 of the array table was determined. Since the amino terminal of refining enzymes was started from the 21st alanine residue of the arrangement of the array number 10 of an array table, The amino acid sequence shown in the array number 10 of an array table is the arrangement of a precursor protein, and it was thought that peptide to the 20th phenylalanine residue was removed from the 1st methionine residue after translation. From this result, it was thought that the amino acid sequence of mature-bodies protein was arrangement shown in the amino acid numbers 1-228 during the arrangement shown in the array number 10 of an array table.

[0051]Example 21 Enterobacter aerogenes IFO 12010 High manifestation Enterobacter aerogenes of the enzyme by change of the promoter sequence of an origin new variant acid phosphatase gene IFO 12010 origin variant acid phosphatase. Site-specific mutation was introduced into the promotor arrangement part of the gene to encode with the gene engineering technique, and the gene which encodes the variant acid phosphatase which the enzyme expression amount increased was built. The gene which introduces variation used plasmid pENP170 built in Example 3 of the Japanese-Patent-Application-No. No. 189226 [12 to] specification. This plasmid is Enterobacter aerogenes. The 1.6kbpDNA fragment cut down with the restriction enzyme SalI containing the gene which encodes IFO 12010 origin variant acid phosphatase, and the restriction enzyme KpnI, It is the plasmid DNA connected with pUC19 (made by TAKARA SHUZO CO., LTD.) cut by SalI and KpnI, and the base sequence of the SalI-KpnI 1.6kbpDNA fragment in pENP170 is arrangement shown in the array number 9 of an array table. The variation introduction to plasmid DNA used the Stratagene quick change site-directed-mutagenesis kit (Quick Change site-directed mutagenesis kit). Oligonucleotide MUT170 for variation introduction compounded using the DNA synthesizer (Applied Biosystem model 394) (array number 99 of an array table), Variation was introduced according to the protocol of Stratagene, using pENP170 as MUT171 (array number 100 of an array table), and a mold. Escherichia coli JM109 (made by TAKARA SHUZO CO., LTD.) was transformed with the conventional method using the obtained plasmid DNA. Plating of this was carried out on L agar medium containing 100 microg/ml ampicillin, and the transformant was obtained. It checked that prepared a plasmid by an alkali bacteriolysis method, determined a base sequence, and the target base was replaced from the transformant. Determination of a base sequence Taq DyeDeoxy Terminator Cycle Sequencing Kit (made by an applied biochemical company) is used, and it is the method of Sanger and others. It carried out according to [journal OBU leakage-at-bulb cue biology (J. Mol. Biol.), the 143rd volume,

and the 161st page (1980)]. It does in this way. Enterobacter aerogenes IFO. The variant gene which encodes the variant gene with which the base sequence of -10 field of the presumed promoter sequence located upstream of 12010 origin presumption acid phosphatase varied to the base sequence of the same TATAAT as the lac promotor of Escherichia coli from AAAAAT was built. The plasmid containing this variant gene was named pENP180.

[0052]Escherichia coli 50 ml of L culture media which contain ampicillin 100microg/ml for Escherichia coli JM109/pENP180 which introduced the gene which changed JM109/pENP170 and -10 field of the promoter sequence, And 50 ml of L culture media containing ampicillin 100microg/ml which added IPTG1mM were inoculated, respectively, and it cultivated at 37 ** for 16 hours. Biomasses were collected by centrifugal separation from the culture medium of each bacillus, and the physiological saline washed once. It was made to react at 30 ** for 1 hour, maintaining [dissolve 15 g/dl of pyrophoric acid and inosine, and 8 g/dl in a 100mM acetic acid buffer (pH 4.0), add so that it may become 100 mg/dl by dry cell weight about each biomass at this, and] pH to 4.0. The quantity of generated 5'-inosinic acid was shown in Table 11. High performance chromatography (HPLC) analyzed inosine and 5'-inosinic acid on condition of the following.

Column: Cosmosil 5C18-AR (4.6x150 mm) [Nacalai Tesque, Inc. products]
mobile phase: -- 5mM potassium phosphate buffer (pH 2.8) / methanol = 95/5 rate-of-flow: -- 1.0 ml/min temperature: -- room temperature detection: -- in UV245nm Escherichia coli JM109/pENP170, although activity was low IPTG additive-free, Even if Escherichia coli JM109/pENP180 did not add IPTG, it showed high activity. Escherichia coli JM109/pENP180 revealed still higher activity by adding IPTG, and it was shown that change of promoterregion is effective.

[0053]
[Table 11]

菌　株	IPTG	生成5'-イノシン酸(g/dl)
エシェリヒア・コリ JM109/pENP170	無添加	0．73
	1mM添加	3．09
エシェリヒア・コリ JM109/pENP180	無添加	2．86
	1mM添加	3．37

[0054]Example 22 Enterobacter aerogenes whose compatibility over a nucleoside improved IFO 12010 Enterobacter aerogenes built in construction example 21 of the origin new variant acid phosphatase gene IFO. Site-specific mutation was introduced into the 12010 origin variant acid phosphatase gene with the gene engineering technique, and the gene which encodes the variant acid phosphatase whose compatibility over a nucleoside, especially guanosine improved was produced. The substitution of amino acid residue was introduced combining the substitution of the amino acid residue identified contributing to the improvement in compatibility with a nucleoside based on the spacial configuration analysis of an Escherichia BURATTAE enzyme. The variation introduction to plasmid DNA used the Stratagene quick change site-directed-mutagenesis kit (Quick Change site-directed mutagenesis kit). 20 kinds of oligonucleotides for variation introduction from MUT180 (array number 101 of an array table) to MUT521 (array number 120 of an array table) were compounded using the DNA synthesizer (Applied Biosystem model 394). According to the protocol of Stratagene, variation was introduced as first mold, using MUT180 and MUT181 as pENP170 and an oligonucleotide for variation introduction. Escherichia coli JM109 (made by TAKARA SHUZO CO., LTD.) was transformed with the conventional method using the obtained plasmid DNA. Plating of this was carried out on L agar medium containing 100 microg/ml ampicillin, and the transformant was obtained. It checked that prepared a plasmid by an alkali bacteriolysis method, determined a base sequence, and the target base was replaced from the transformant. Determination of a base sequence In accordance with the method (the above-mentioned academic journal) of Sanger and others, it carried out using Taq DyeDeoxy Terminator Cycle Sequencing Kit (made by an applied biochemical company). Thus, the 153rd threonine residue (ACC) built the gene which encodes the variant acid phosphatase replaced by serine residue (TCC), and named pENP200 the plasmid containing this variant gene. The operation same as a new mold was repeated for the plasmid which introduced variation, and site-specific mutation was introduced

cumulatively. It checked that prepared a plasmid by an alkali bacteriolysis method, determined a base sequence, and the target base was replaced from the transformant. The variant enzyme gene and mutation site which encode the created variant acid phosphatase were shown in Table 12. The amino acid residue of the mutation site shows the amino acid residue in the amino acid sequence of the maturation protein shown in the array number 10 of the array table.

[0055]Each variant acid phosphatase gene. The included plasmid. introduced Escherichia coli . JM109/pENP180, Escherichia coli JM109/pENP320, Escherichia coli JM109/pENP340, Escherichia coli JM109/pENP410, Escherichia coli JM109/pENP510, And Escherichia coli JM109/pENP520 was inoculated into 50 ml of L culture media containing ampicillin 100microg/ml and IPTG1mM, and it cultivated at 37 ** for 16 hours. The biomass was suspended to a 50-ml 100mM phosphoric acid buffer (pH 7.0), ultrasonication was performed for 20 minutes at 4 **, and the biomass was crushed. Biomasses were collected by centrifugal separation from the culture medium of each bacillus, and the physiological saline washed once. The treating solution was centrifuged and the cell-free extract was prepared except for the insoluble fraction. Km value to the inosine and guanosine in a phosphotransfer reaction was measured using each cell-free extract.

[0056]Measurement of the phosphate transfer activity to a nucleoside was performed by using inosine and guanosine as a substrate on the following conditions. pH 4.0 was performed by the reaction mixture (1 ml) containing the inosine or the guanosine, 100 micro a mol [/ml] sodium pyrophosphate, the 100 micro mol/ml sodium acetate buffer solution (pH 4.0), and the enzyme of various concentration, and the reaction was performed at 30 ** for 10 minutes. After adding 2N chloride 200mul and suspending a reaction, under [a fixed quantity / which was generated by the phosphotransfer reaction except for precipitate by centrifugal separation / 5'-inosinic acid or 5'-guanylic acid]. High performance chromatography (HPLC) analyzed inosine, guanosine, 5'-inosinic acid, and 5'-guanylic acid on the same conditions as Example 21. The concentration of inosine or guanosine is changed on the reaction condition of the above-mentioned presentation, phosphate transfer activity is measured, and it is a Hanes-Woolf plot. It asked for the kinetic constant of the inosine in a phosphotransfer reaction, and guanosine by [a ZABAIO chemical journal (Biochem.J.), the 26th volume, and the 1406th page (1932)]. The result was shown in Table 13 - 16. It became clear that Km value to guanosine falls notably and the compatibility of Km value of the enzyme variant created in the example over guanosine is improving. Km value [as opposed to inosine in four kinds of enzyme variants other than the enzyme variant by which a code is carried out to pENP520] was also falling dramatically.

[0057]

[Table 12]

配列の名称	配列番号		長さ	配列
MUT170	99	センス	30	5'-CTT ACA GAT GAC **TAT AAT** GTG ACT AAA AAC
MUT171	100	アンチセンス	30	5'-GTT TTT AGT CAC ATT ATA GTC ATC TGT AAG
MUT180	101	センス	33	5'-TCT ACC GGT TGG GCA **TCC** GCG CTG GTA CTG GCG
MUT181	102	アンチセンス	33	5'-CGC CAG TAC CAG CGC GGA TGC CCA ACC GGT AGA
MUT300	103	センス	33	5'-TCC GGC CAT ACC TCT **TCC** GGT TGG GCA TCC GCG
MUT301	104	アンチセンス	33	5'-CGC GGA TGC CCA ACC GGA AGA GGT ATG GCC GGA
MUT310	105	センス	33	5'-GAT GCT GAC CTG GCC **GTT** GGC GAC GTC GCG AAT
MUT311	106	アンチセンス	33	5'-ATT CGC GAC GTC GCC AAC GGC CAG GTC AGC ATC
MUT320	107	センス	33	5'-CTG ACA AAT ATG ATT **CTG** GAT GCC GGC GAT CTG
MUT321	108	アンチセンス	33	5'-CAG ATC GCC GGC ATC CAG AAT CAT ATT TGT CAG
MUT330	109	センス	33	5'-GAT GCT GAC CTG GCC **ATG** GGC GAC GTC GCG AAT
MUT331	110	アンチセンス	33	5'-ATT CGC GAC GTC GCC CAT GGC CAG GTC AGC ATC
MUT340	111	センス	33	5'-CTG ACA AAT ATG ATT **CAG** GAT GCC GGC GAT CTG
MUT341	112	アンチセンス	33	5'-CAG ATC GCC GGC ATC CTG AAT CAT ATT TGT CAG
MUT400	113	センス	33	5'-TCC GGC CAT ACC TCT **GCT** GGT TGG GCA TCC GCG
MUT401	114	アンチセンス	33	5'-CGC GGA TGC CCA ACC AGC AGA GGT ATG GCC GGA
MUT500	115	センス	33	5'-TCC GGC CAT ACC TCT **GGC** GGT TGG GCA TCC GCG
MUT501	116	アンチセンス	33	5'-CGC GGA TGC CCA ACC GCC AGA GGT ATG GCC GGA
MUT510	117	センス	33	5'-GAT GCT GAC CTG GCC **GAA** GGC GAC GTC GCG AAT
MUT511	118	アンチセンス	33	5'-ATT CGC GAC GTC GCC TTC GGC CAG GTC AGC ATC
MUT520	119	センス	33	5'-GAT GCT GAC CTG GCC **AAA** GGC GAC GTC GCG AAT
MUT521	120	アンチセンス	33	5'-ATT CGC GAC GTC GCC TTT GGC CAG GTC AGC ATC

[0058]
[Table 13]

プラスミド名	変異を導入したプラスミド	変異導入に用いたプライマー	変異点及びアミノ酸置換
pENP180			61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 151I(ATC)→T(ACC)
pENP200	pENP130	MUT180, MUT181	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 151T(ACC)→S(TCC)
pENP300	pENP200	MUT300, MUT301	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 151I(ATC)→T(ACC) 149T(ACC)→S(TCC) 151T(ACC)→S(TCC)
pENP310	pENP300	MUT310, MUT311	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 70A(GCC)→V(GTT) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 151I(ATC)→T(ACC) 149T(ACC)→S(TCC) 151T(ACC)→S(TCC)
pENP320	pENP310	MUT320, MUT321	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC)

[0059]
[Table 14]

			69S(AGC)→A(GCC) 70A(GCC)→V(GTT) 72G(GGC)→D(GAC) 102E(GAG)→L(CTG) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→S(TCC) 151T(ACC)→S(TCC)
pENP330	pENP300	MUT330, MUT331	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 70A(GCC)→M(ATG) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→S(TCC) 151T(ACC)→S(TCC)
pENP340	pENP330	MUT340, MUT341	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 70A(GCC)→V(GTT) 72G(GGC)→D(GAC) 102E(GAG)→Q(CAG) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→S(TCC) 151T(ACC)→S(TCC)
pENP400	pENP200	MUT400, MUT401	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→A(GCT) 151T(ACC)→S(TCC)
pENP410	pENP400	MUT310, MUT311	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 70A(GCC)→V(GTT) 72G(GGC)→D(GAC)

[0060]
[Table 15]

			133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→A(GCT) 151T(ACC)→S(TCC)
pENP500	pENP200	MUT500, MUT501	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→G(GGC) 151T(ACC)→S(TCC)
pENP510	pENP500	MUT510, MUT511	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 70A(GCC)→E(GAA) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→G(GGC) 151T(ACC)→S(TCC)
pENP520	pENP500	MUT520, MUT521	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 70A(GCC)→K(AAA) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→G(GGC) 151T(ACC)→S(TCC)

[0061]
[Table 16]

	イノシンに対するKm値 (mM)	イノシンを基質とした場合の相対活性	グアノシンに対するKm値 (mM)	グアノシンを基質とした時の相対活性
pENP180	40	1.0	40	1.0
pENP320	19	1.9	4.6	1.5
pENP340	19	1.4	5.1	1.3
pENP410	18	1.0	4.9	0.70
pENP510	17	0.55	4.0	0.39
pENP520	46	0.63	4.4	0.21

[0062]Example 23 Enterobacter aerogenes whose compatibility over guanosine improved IFO 12010 the variant acid phosphatase gene of each phosphorylation of the guanosine by an origin new variant acid phosphatase transgenics bacillus. The included plasmid. introduced Escherichia coli . JM109/pENP180, Escherichia coli JM109/pENP320, Escherichia coli JM109/pENP340, Escherichia coli JM109/pENP410, Escherichia coli JM109/pENP510, And Escherichia coli JM109/pENP520 was inoculated into 50 ml of L culture media containing ampicillin 100microg/ml and IPTG1mM, and it cultivated at 37 ** for 16 hours. 10 g/dl of pyrophoric acid and 6.6 g/dl of grinding guanosine prepared like Example 1 of JP,12-189226,A are dissolved in a 100mM acetic acid buffer (pH 4.5), It

was made to react at 35 ** for 12 hours, maintaining [to this, add each biomass so that it may become 100 mg/dl by dry cell weight, and] pH to 4.5. The quantity of generated 5'-guanylic acid was shown in Table 17. Escherichia coli each whose bacillus which introduced the enzyme variant as shown in Table 17 is an old stock Productivity improved compared with JM109/pENP180, and generation accumulation of the 5'-guanylic acid was carried out with high yield.

[0063]

[Table 17]

菌　株	生成 5'-グアニル酸 (g/dl)
エシェリヒア・コリ JM109/pENP180	9．90
エシェリヒア・コリ JM109/pENP320	10．4
エシェリヒア・コリ JM109/pENP340	10．2
エシェリヒア・コリ JM109/pENP410	11．1
エシェリヒア・コリ JM109/pENP510	11．0
エシェリヒア・コリ JM109/pENP520	10．5

[0064]

[Effect of the Invention]As explained to details above, according to this invention, a variant nucleoside 5'-phosphoric acid production enzyme whose nucleoside 5'-phosphoric acid productivity improved, and a manufacturing method for the same are provided. According to this invention, the microorganism which holds the recombinant DNA which contains in the manufacturing method of nucleoside 5'-phosphoric acid the gene which encodes the useful aforementioned enzyme variant, and this gene, and this recombinant DNA is provided. It succeeded in the break through of the proteinic new spacial configuration by X ray crystal-structure-analysis art.

[0065]

[Layout Table]

<110> Ajinomoto Co., Inc. (Ajinomoto Co., Inc.)

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[Translation done.]

* NOTICES *

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1]It is a figure showing these space position relations with five amino acid residue used as the component of the active site which has phosphatase activity or phosphoryl-group-transfer activity as a distance between Calpha atoms.
[Drawing 2]It is the figure which aligned the amino acid sequence of EB-AP with the amino acid sequence of Morganella MORUGANI, Salmonella typhimurium, and ZAIMO monas MOBIRISU origin acid phosphatase.
[Drawing 3]It is a photograph of the computer graphics (CG) which shows the crystal structure of the bond form model of an EB-AP reaction intermediate analog and inosine.
[Drawing 4]It is a photograph of CG which shows the crystal structure of the hexamer molecule of EB-AP.
[Drawing 5]It is a photograph of CG which shows the crystal structure of the subunit of EB-AP.
[Drawing 6]It is a figure showing the active site architecture of EB-AP.
[Drawing 7]It is a figure showing the primer set used for the site-directed-mutagenesis method.
[Drawing 8]It is a figure showing the primer set used for the site-directed-mutagenesis method.
[Drawing 9]It is a figure showing the result of having performed amino acid sequence alignment of EB-AP and Enterobacter aerogenes origin acid phosphatase (EA-AP) by the program BLAST.
[Drawing 10]It is a figure showing the crystallography data (1) of the structure of EB-AP.
[Drawing 11]It is a figure showing the crystallography data (2) of the structure of EB-AP.
[Drawing 12]It is a figure showing the crystallography data (3) of the structure of EB-AP.
[Drawing 13]It is a figure showing the crystallography data (4) of the structure of EB-AP.
[Drawing 14]It is a figure showing the crystallography data (5) of the structure of EB-AP.
[Drawing 15]It is a figure showing the crystallography data (6) of the structure of EB-AP.
[Drawing 16]It is a figure showing the crystallography data (7) of the structure of EB-AP.
[Drawing 17]It is a figure showing the crystallography data (8) of the structure of EB-AP.
[Drawing 18]It is a figure showing the crystallography data (9) of the structure of EB-AP.
[Drawing 19]It is a figure showing the crystallography data (10) of the structure of EB-AP.
[Drawing 20]It is a figure showing the crystallography data (11) of the structure of EB-AP.
[Drawing 21]It is a figure showing the crystallography data (12) of the structure of EB-AP.
[Drawing 22]It is a figure showing the crystallography data (13) of the structure of EB-AP.
[Drawing 23]It is a figure showing the crystallography data (14) of the structure of EB-AP.
[Drawing 24]It is a figure showing the crystallography data (15) of the structure of EB-AP.
[Drawing 25]It is a figure showing the crystallography data (16) of the structure of EB-AP.
[Drawing 26]It is a figure showing the crystallography data (17) of the structure of EB-AP.
[Drawing 27]It is a figure showing the crystallography data (18) of the structure of EB-AP.
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[Drawing 32]It is a figure showing the crystallography data (23) of the structure of EB-AP.
[Drawing 33]It is a figure showing the crystallography data (24) of the structure of EB-AP.

[Drawing 34]It is a figure showing the crystallography data (25) of the structure of EB-AP.
[Drawing 35]It is a figure showing the crystallography data (26) of the structure of EB-AP.
[Drawing 36]It is a figure showing the crystallography data (27) of the structure of EB-AP.
[Drawing 37]It is a figure showing the crystallography data (28) of the structure of EB-AP.
[Drawing 38]It is a figure showing the crystallography data (29) of the structure of EB-AP.
[Drawing 39]It is a figure showing the crystallography data (30) of the structure of EB-AP.
[Drawing 40]It is a figure showing the crystallography data (31) of the structure of EB-AP.
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[Drawing 43]It is a figure showing the crystallography data (34) of the structure of EB-AP.
[Drawing 44]It is a figure showing the crystallography data (35) of the structure of EB-AP.
[Drawing 45]It is a figure showing the crystallography data (36) of the structure of EB-AP.
[Drawing 46]It is a figure showing the primer set used for the site-directed-mutagenesis method.
[Drawing 47]It is a figure showing the primer set used for the site-directed-mutagenesis method.
[Drawing 48]It is a figure showing the primer set used for the site-directed-mutagenesis method.

[Translation done.]

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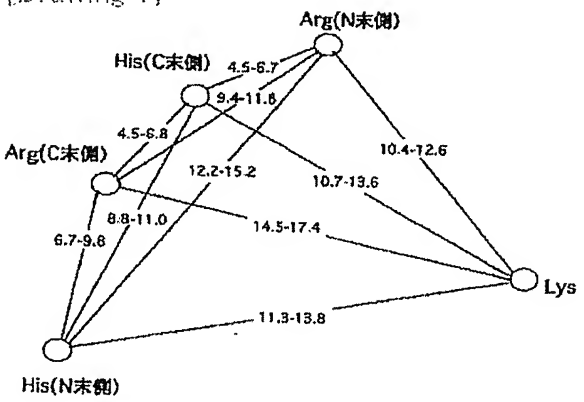
DRAWINGS

[Drawing 1]



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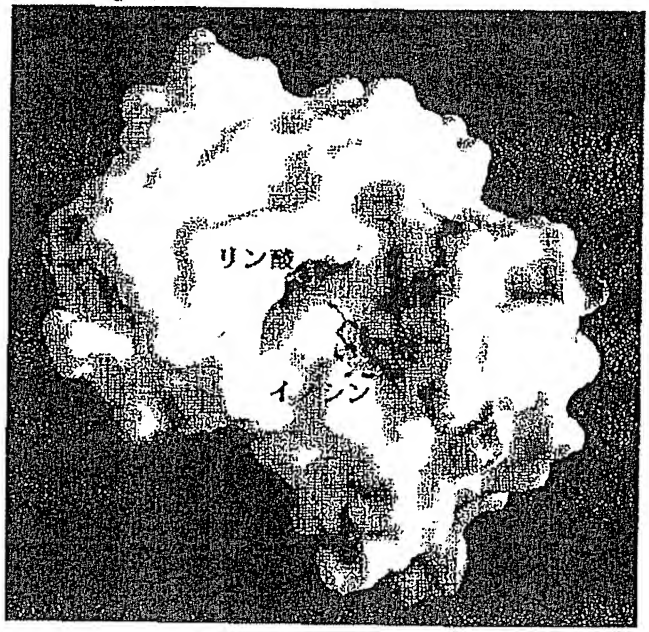

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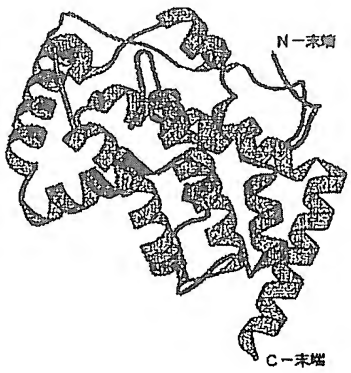

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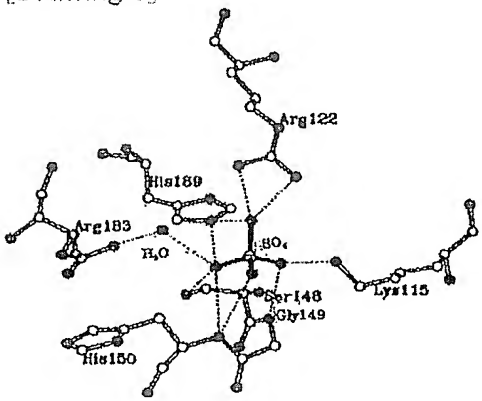

[Drawing 2]

α1
α2
α3
E.blattae :LALVATGNDT TTKPDLYYLK NSEAINSLAL LPPPAVGSI AFLNDQAMYE QGRLLRNTER GKLAAEDANL 70
M.morganii : AIPAGNDA TTKPDLYYLK NEQAIDSLKL LPPPEVGSI QFLNDQAMYE KGRMLRNTER GKQAQADADL
S.typhimurium : KYT SAETVQPFHS PEESVNSQFY LPPPPGNDDP AYRYDKEAYF KGYAILGSPR WKQAAEDADV
Z.mobilis :ASGLSQSVSA HTEKSEPSST YHFHSDPLLY LAPPPTSGSP LQAHDDQTFN STRQLKGSTR WALATQDADL
α4
α5
α6
α7
α8
α9
E.blattae :SSGGVANAFS GAFGSPITEK DAPALHKLLT NMIEDAGDLA TRSAKDHTMR IRPFAFYGVS TCNTTEQDRL 140
M.morganii :AAGGVATAFS GAFGYPITEK DSPELYKLLT NMIEDAGDLA TRSAKEHTMR IRPFAFYGTE TCNTKDQKKL
S.typhimurium :SVENIARIFS PVVGAKINPK DTPETWAMLK NLITMGGYYA TASAKKYTMR TRPFVLFNHS TCRPEDENTL
Z.mobilis :HLASVLKDYA CAAGMNLDIA QLPHLANLIK RALRTEYDDI GR-AKNNWNR KRPFVDTDQP ICTEKDREGL
α10
α11
α12
E.blattae :SKNGSYPSGH TSIGWATALV LAEINPQRQN EILKRGYELG QSRVICGYHW QSDVDAARVV GSAVVATLHT 210
M.morganii :STNGSYPSGH TSIGWATALV LAEVNPANQD AILERGYQLG QSRVICGYHW QSDVDAARIV GSAAVATLHS
S.typhimurium :RKNGSYPSGH TAYGTLIALV LSEARPERAQ ELARRGWEFG QSRVICGAHW QSDVDAGRYV GAVEFARLQT
Z.mobilis :GKQGSYPSGH TTIGWSVALI LAELIPDHAA NILQRGQIFG TSRTVCGAHW FSDVQAGYIM ASGEIAALHG
α13
E.blattae :NPAFQQQLQK AKAEFAQHQK K
M.morganii :DPAFQAQLAK AKQEFAQKSQ K
S.typhimurium :IPAFQKSLAK VREELNDKNN LLSKEDHPKL NY
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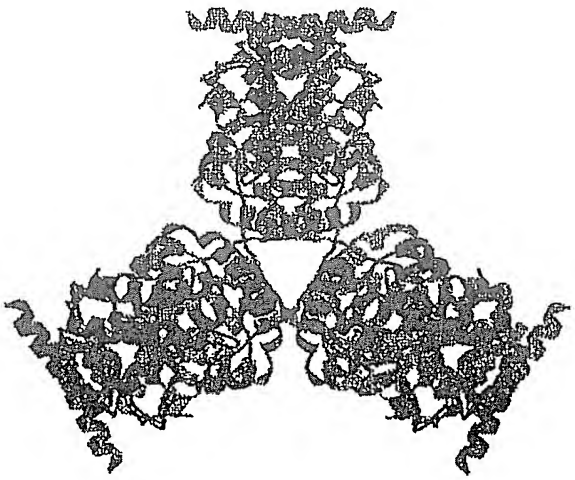
[Drawing 4]



[Drawing 7]

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S72F(s)    5'-CA-AAC-CTG-AGC-TTT-GGC-GAT-GTG-GC-3'
S72F(as)   3'-GT-TTG-GAC-TCG-AAA-CCG-CTA-CAC-CG-5'
                 N   L   S   F72  G   D   V
S72Y(s)    5'-CA-AAC-CTG-AGC-TAC-GGC-GAT-GTG-GC-3'
S72Y(as)   3'-GT-TTG-GAC-TCG-ATG-CCG-CTA-CAC-CG-5'
                 N   L   S   Y72  G   D   V
S72W(s)    5'-CA-AAC-CTG-AGC-TGG-GGC-GAT-GTG-GC-3'
S72W(as)   3'-GT-TTG-GAC-TCG-ACC-CCG-CTA-CAC-CG-5'
                 N   L   S   W72  G   D   V
S72D(s)    5'-CA-AAC-CTG-AGC-GAC-GGC-GAT-GTG-GC-3'
S72D(as)   3'-GT-TTG-GAC-TCG-CTG-CCG-CTA-CAC-CG-5'
                 N   L   S   D72  G   D   V
S72V(s)    5'-CA-AAC-CTG-AGC-GTT-GGC-GAT-GTG-GC-3'
S72V(as)   3'-GT-TTG-GAC-TCG-CAA-CCG-CTA-CAC-CG-5'
                 N   L   S   V72  G   D   V
S72E(s)    5'-CA-AAC-CTG-AGC-GAA-GGC-GAT-GTG-GC-3'
S72E(as)   3'-GT-TTG-GAC-TCG-CTT-CCG-CTA-CAC-CG-5'
                 N   L   S   E72  G   D   V
S72M(s)    5'-CA-AAC-CTG-AGC-ATG-GGC-GAT-GTG-GC-3'
S72M(as)   3'-GT-TTG-GAC-TCG-TAC-CCG-CTA-CAC-CG-5'
                 N   L   S   M72  G   D   V
S72T(s)    5'-CA-AAC-CTG-AGC-ACC-GGC-GAT-GTG-GC-3'
S72T(as)   3'-GT-TTG-GAC-TCG-TGG-CCG-CTA-CAC-CG-5'
                 N   L   S   T72  G   D   V
S72L(s)    5'-CA-AAC-CTG-AGC-CTG-GGC-GAT-GTG-GC-3'
S72L(as)   3'-GT-TTG-GAC-TCG-GAC-CCG-CTA-CAC-CG-5'
                 N   L   S   L72  G   D   V
S72R(s)    5'-CA-AAC-CTG-AGC-CGT-GGC-GAT-GTG-GC-3'
S72R(as)   3'-GT-TTG-GAC-TCG-GCA-CCG-CTA-CAC-CG-5'
                 N   L   S   R72  G   D   V
S72Q(s)    5'-CA-AAC-CTG-AGC-CAG-GGC-GAT-GTG-GC-3'
S72Q(as)   3'-GT-TTG-GAC-TCG-GTC-CCG-CTA-CAC-CG-5'
                 N   L   S   Q72  G   D   V
S72K(s)    5'-CA-AAC-CTG-AGC-AAA-GGC-GAT-GTG-GC-3'
S72K(as)   3'-GT-TTG-GAC-TCG-TTT-CCG-CTA-CAC-CG-5'
                 N   L   S   K72  G   D   V
S72P(s)    5'-CA-AAC-CTG-AGC-CCG-GGC-GAT-GTG-GC-3'
S72P(as)   3'-GT-TTG-GAC-TCG-GGC-CCG-CTA-CAC-CG-5'
                 N   L   S   P72  G   D   V
S72A(s)    5'-CA-AAC-CTG-AGC-GCG-GGC-GAT-GTG-GC-3'
S72A(as)   3'-GT-TTG-GAC-TCG-CGC-CCG-CTA-CAC-CG-5'
                 N   L   S   A72  G   D   V
S72N(s)    5'-CA-AAC-CTG-AGC-AAC-GGC-GAT-GTG-GC-3'
S72N(as)   3'-GT-TTG-GAC-TCG-TTG-CCG-CTA-CAC-CG-5'
                 N   L   S   N72  G   D   V
S72G(s)    5'-CA-AAC-CTG-AGC-GGT-GGC-GAT-GTG-GC-3'
S72G(as)   3'-GT-TTG-GAC-TCG-CCA-CCG-CTA-CAC-CG-5'
                 N   L   S   G72  G   D   V
S72H(s)    5'-CA-AAC-CTG-AGC-CAC-GGC-GAT-GTG-GC-3'
S72H(as)   3'-GT-TTG-GAC-TCG-GTG-CCG-CTA-CAC-CG-5'
                 N   L   S   H72  G   D   V
```

[Drawing 8]

```
L16W(s)     5'-CG-AAA-CCG-GAT-TGG-TAC-TAC-CTC-AA-3'
L16W(as)    3'-GC-TTT-GGC-CTA-ACC-ATG-ATG-GAG-TT-5'
                    K    P    D    W16  Y    Y    L

S71W(s)     5'-AT-GCA-AAC-CTG-TGG-AGT-GGC-GAT-GT-3'
S71W(as)    3'-TA-CGT-TTG-GAC-ACC-TCA-CCG-CTA-CA-5'
                    A    N    L    W71  S    G    D

G73W(s)     5'-AC-CTG-AGC-AGT-TGG-GAT-GTG-GCG-AA-3'
G73W(as)    3'-TG-GAC-TCG-TCA-ACC-CTA-CAC-CGC-TT-5'
                    L    S    S    W73  D    V    A

E104F(s)    5'-CC-AAT-ATG-ATT-TTT-GAC-GCC-GGG-GA-3'
E104F(as)   3'-GG-TTA-TAC-TAA-AAA-CTG-CGG-CCC-CT-5'
                    N    M    I    F104 D    A    G

E104W(s)    5'-CC-AAT-ATG-ATT-TGG-GAC-GCC-GGG-GA-3'
E104W(as)   3'-GG-TTA-TAC-TAA-ACC-CTG-CGG-CCC-CT-5'
                    N    M    I    W104 D    A    G
```

[Drawing 9]

```
EB-AP:      LALVATGNDTTTKPDLYYLKNSEAINSLALLPPPPAVGSIAFLNDQAMYEQGRLLRNTER
               V  GND TTKPDLYYLKN++AI+SLALLPPPP VGSIAFLNDQAMYE+GRLLRNTER
EA-AP:        LVPAGNDATTKPDLYYLKNAQAIDSLALLPPPPEVGSIAFLNDQAMYEKGRLLRNTER

                        [72]
EB-AP:      GKLAAEDANLSSGGVANAFSGAFGSPITEKDAPALHKLLTNMIEDAGDLATRSAKDHYMR
            GKLAAEDANLS+GGVANAFS AFGSPITEKDAP LHKLLTNMIEDAGDLATRSAK+ YMR
EA-AP:      GKLAAEDANLSAGGVANAFSSAFGSPITEKDAPQLHKLLTNMIEDAGDLATRSAKEKYMR
                        [70]

EB-AP:      IRPFAFYGVSTCNTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELG
            IRPFAFYGVSTCNTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELG
EA-AP:      IRPFAFYGVSTCNTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELG

EB-AP:      QSRVICGYHWQSDVDAARVVGSAVVATLHTNPAFQQQLQKAKAEFAQHQKK
            +SRVICGYHWQSDVDAAR+VGSAVVATLHTNPAFQQQLQKAK EFA+ QK
EA-AP:      ESRVICGYHWQSDVDAARIVGSAVVATLHTNPAFQQQLQKAKDEFAKTQK
```

[Drawing 10]

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ATOM      1  N   GLY A   7      35.965  71.208  89.712  1.00 36.57
ATOM      2  CA  GLY A   7      37.459  71.295  89.574  1.00 31.92
ATOM      3  C   GLY A   7      38.160  69.982  89.872  1.00 29.76
ATOM      4  O   GLY A   7      39.301  69.858  89.492  1.00 31.81
ATOM      5  N   ASN A   8      37.485  68.990  90.532  1.00 26.40
ATOM      6  CA  ASN A   8      38.284  67.775  90.697  1.00 26.63
ATOM      7  C   ASN A   8      38.466  67.018  89.396  1.00 29.21
ATOM      8  O   ASN A   8      37.736  67.238  88.431  1.00 30.52
ATOM      9  CB  ASN A   8      37.677  66.810  91.702  1.00 27.01
ATOM     10  CG  ASN A   8      37.725  67.396  93.104  1.00 32.45
ATOM     11  OD1 ASN A   8      38.751  67.744  93.636  1.00 30.02
ATOM     12  ND2 ASN A   8      36.545  67.536  93.707  1.00 31.60
ATOM     13  N   ASP A   9      39.455  66.154  89.463  1.00 29.14
ATOM     14  CA  ASP A   9      39.787  65.216  88.391  1.00 30.47
ATOM     15  C   ASP A   9      40.661  64.081  88.901  1.00 31.02
ATOM     16  O   ASP A   9      40.804  63.931  90.110  1.00 31.00
ATOM     17  CB  ASP A   9      40.394  65.960  87.195  1.00 30.92
ATOM     18  CG  ASP A   9      41.802  66.484  87.429  1.00 32.66
ATOM     19  OD1 ASP A   9      42.307  66.333  88.532  1.00 35.03
ATOM     20  OD2 ASP A   9      42.400  67.018  86.493  1.00 31.63
ATOM     21  N   THR A  10      41.272  63.298  87.998  1.00 28.72
ATOM     22  CA  THR A  10      42.188  62.228  88.430  1.00 28.53
ATOM     23  C   THR A  10      43.408  62.655  89.259  1.00 30.10
ATOM     24  O   THR A  10      43.946  61.944  90.095  1.00 29.06
ATOM     25  CB  THR A  10      42.692  61.405  87.235  1.00 26.05
ATOM     26  OG1 THR A  10      43.272  60.172  87.655  1.00 27.75
ATOM     27  CG2 THR A  10      43.670  62.174  86.313  1.00 23.76
ATOM     28  N   THR A  11      43.814  63.900  88.996  1.00 30.82
ATOM     29  CA  THR A  11      44.932  64.389  89.799  1.00 32.79
ATOM     30  C   THR A  11      44.605  64.736  91.267  1.00 36.32
ATOM     31  O   THR A  11      45.435  64.658  92.162  1.00 37.21
ATOM     32  CB  THR A  11      45.588  65.591  89.143  1.00 30.53
ATOM     33  OG1 THR A  11      44.845  66.781  89.359  1.00 27.79
ATOM     34  CG2 THR A  11      45.899  65.362  87.656  1.00 32.16
ATOM     35  N   THR A  12      43.317  65.076  91.495  1.00 34.81
ATOM     36  CA  THR A  12      42.910  65.213  92.900  1.00 32.91
ATOM     37  C   THR A  12      42.265  63.992  93.549  1.00 33.08
ATOM     38  O   THR A  12      42.350  63.742  94.736  1.00 32.49
ATOM     39  CB  THR A  12      41.963  66.395  93.077  1.00 30.92
ATOM     40  OG1 THR A  12      40.719  66.162  92.409  1.00 32.04
ATOM     41  CG2 THR A  12      42.599  67.667  92.543  1.00 29.75
ATOM     42  N   LYS A  13      41.565  63.229  92.703  1.00 31.17
ATOM     43  CA  LYS A  13      40.791  62.064  93.174  1.00 30.27
ATOM     44  C   LYS A  13      40.904  60.812  92.287  1.00 31.40
ATOM     45  O   LYS A  13      39.981  60.348  91.605  1.00 33.05
ATOM     46  CB  LYS A  13      39.294  62.395  93.331  1.00 29.09
ATOM     47  CG  LYS A  13      39.001  63.747  93.965  1.00 32.97
ATOM     48  CD  LYS A  13      37.536  64.076  94.166  1.00 37.86
ATOM     49  CE  LYS A  13      36.767  62.909  94.772  1.00 47.28
ATOM     50  NZ  LYS A  13      35.340  63.270  94.947  1.00 52.08
ATOM     51  N   PRO A  14      42.138  60.283  92.279  1.00 33.01
ATOM     52  CA  PRO A  14      42.516  59.249  91.290  1.00 32.06
ATOM     53  C   PRO A  14      41.823  57.907  91.452  1.00 30.98
ATOM     54  O   PRO A  14      41.961  56.989  90.668  1.00 32.57
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[Drawing 11]

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ATOM     55  CB  PRO A  14      44.035  59.145  91.468  1.00 34.46
ATOM     56  CG  PRO A  14      44.283  59.564  92.920  1.00 33.02
ATOM     57  CD  PRO A  14      43.225  60.638  93.181  1.00 34.46
ATOM     58  N   ASP A  15      41.046  57.815  92.513  1.00 29.27
ATOM     59  CA  ASP A  15      40.204  56.655  92.809  1.00 28.89
ATOM     60  C   ASP A  15      38.810  56.684  92.146  1.00 21.76
ATOM     61  O   ASP A  15      38.078  55.706  92.030  1.00 20.59
ATOM     62  CB  ASP A  15      40.125  56.599  94.368  1.00 37.60
ATOM     63  CG  ASP A  15      39.589  57.903  95.080  1.00 45.11
ATOM     64  OD1 ASP A  15      40.062  59.044  94.817  1.00 45.67
ATOM     65  OD2 ASP A  15      38.687  57.751  95.922  1.00 49.07
ATOM     66  N   LEU A  16      38.495  57.910  91.726  1.00 20.49
ATOM     67  CA  LEU A  16      37.182  58.179  91.135  1.00 23.90
ATOM     68  C   LEU A  16      37.156  58.814  89.727  1.00 22.23
ATOM     69  O   LEU A  16      36.109  59.011  89.134  1.00 23.21
ATOM     70  CB  LEU A  16      36.354  59.099  92.029  1.00 23.35
ATOM     71  CG  LEU A  16      35.814  58.432  93.297  1.00 25.48
ATOM     72  CD1 LEU A  16      34.876  57.253  93.075  1.00 24.05
ATOM     73  CD2 LEU A  16      35.092  59.477  94.104  1.00 25.22
ATOM     74  N   TYR A  17      38.343  59.175  89.273  1.00 20.96
ATOM     75  CA  TYR A  17      38.555  59.605  87.889  1.00 22.04
ATOM     76  C   TYR A  17      39.780  58.903  87.334  1.00 22.80
ATOM     77  O   TYR A  17      40.790  58.799  88.021  1.00 23.48
ATOM     78  CB  TYR A  17      38.856  61.095  87.711  1.00 18.01
ATOM     79  CG  TYR A  17      37.928  62.099  88.371  1.00 24.78
ATOM     80  CD1 TYR A  17      37.129  62.916  87.542  1.00 22.78
ATOM     81  CD2 TYR A  17      37.905  62.248  89.781  1.00 23.58
ATOM     82  CE1 TYR A  17      36.317  63.919  88.113  1.00 26.51
ATOM     83  CE2 TYR A  17      37.090  63.240  90.349  1.00 22.88
ATOM     84  CZ  TYR A  17      36.303  64.059  89.517  1.00 24.63
ATOM     85  OH  TYR A  17      35.482  65.023  90.066  1.00 22.92
ATOM     86  N   TYR A  18      39.670  58.482  86.053  1.00 26.17
ATOM     87  CA  TYR A  18      40.838  58.209  85.191  1.00 21.13
ATOM     88  C   TYR A  18      41.332  59.414  84.464  1.00 19.92
ATOM     89  O   TYR A  18      42.490  59.511  84.083  1.00 22.64
ATOM     90  CB  TYR A  18      40.563  57.195  84.080  1.00 17.53
ATOM     91  CG  TYR A  18      40.312  55.826  84.610  1.00 16.91
ATOM     92  CD1 TYR A  18      41.425  55.028  84.916  1.00 19.86
ATOM     93  CD2 TYR A  18      38.985  55.372  84.771  1.00 16.65
ATOM     94  CE1 TYR A  18      41.218  53.725  85.383  1.00 18.64
ATOM     95  CE2 TYR A  18      38.765  54.053  85.213  1.00 17.52
ATOM     96  CZ  TYR A  18      39.892  53.262  85.515  1.00 21.18
ATOM     97  OH  TYR A  18      39.734  51.974  85.977  1.00 26.15
ATOM     98  N   LEU A  19      40.412  60.336  84.236  1.00 21.49
ATOM     99  CA  LEU A  19      40.788  61.462  83.366  1.00 22.71
ATOM    100  C   LEU A  19      41.094  62.812  84.021  1.00 25.01
ATOM    101  O   LEU A  19      40.771  63.125  85.159  1.00 25.24
ATOM    102  CB  LEU A  19      39.708  61.669  82.290  1.00 21.68
ATOM    103  CG  LEU A  19      39.301  60.442  81.432  1.00 22.88
ATOM    104  CD1 LEU A  19      40.430  59.842  80.583  1.00 20.39
ATOM    105  CD2 LEU A  19      38.078  60.812  80.608  1.00 18.83
ATOM    106  N   LYS A  20      41.736  63.667  83.246  1.00 26.44
ATOM    107  CA  LYS A  20      41.947  65.032  83.717  1.00 26.77
ATOM    108  C   LYS A  20      40.935  66.034  83.292  1.00 26.42
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[Drawing 12]

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ATOM    109  O   LYS A  20      40.182  65.870  82.341  1.00 29.05
ATOM    110  CB  LYS A  20      43.239  65.608  83.187  1.00 30.61
ATOM    111  CG  LYS A  20      44.400  64.791  83.648  1.00 32.90
ATOM    112  CD  LYS A  20      45.633  65.326  82.963  1.00 39.72
ATOM    113  CE  LYS A  20      46.698  64.259  83.113  1.00 50.27
ATOM    114  NZ  LYS A  20      46.148  62.977  82.610  1.00 62.00
ATOM    115  N   ASN A  21      41.050  67.184  83.943  1.00 24.09
ATOM    116  CA  ASN A  21      40.154  68.246  83.530  1.00 23.98
ATOM    117  C   ASN A  21      40.177  68.539  82.032  1.00 25.08
ATOM    118  O   ASN A  21      39.134  68.722  81.427  1.00 25.36
ATOM    119  CB  ASN A  21      40.310  69.512  84.371  1.00 23.81
ATOM    120  CG  ASN A  21      39.601  69.311  85.697  1.00 26.97
ATOM    121  OD1 ASN A  21      38.392  69.175  85.836  1.00 26.36
ATOM    122  ND2 ASN A  21      40.403  69.303  86.744  1.00 32.00
ATOM    123  N   SER A  22      41.378  68.486  81.450  1.00 23.72
ATOM    124  CA  SER A  22      41.592  68.804  80.008  1.00 25.53
ATOM    125  C   SER A  22      40.992  67.752  79.068  1.00 25.77
ATOM    126  O   SER A  22      40.524  68.007  77.966  1.00 27.65
ATOM    127  CB  SER A  22      43.079  68.868  79.699  1.00 23.34
ATOM    128  OG  SER A  22      43.719  67.716  80.303  1.00 33.30
ATOM    129  N   GLU A  23      40.957  66.529  79.624  1.00 22.35
ATOM    130  CA  GLU A  23      40.320  65.466  78.899  1.00 21.87
ATOM    131  C   GLU A  23      38.811  65.375  78.974  1.00 23.18
ATOM    132  O   GLU A  23      38.197  64.451  78.471  1.00 25.83
ATOM    133  CB  GLU A  23      40.923  64.165  79.337  1.00 22.13
ATOM    134  CG  GLU A  23      42.451  64.215  79.214  1.00 26.78
ATOM    135  CD  GLU A  23      43.021  62.908  79.718  1.00 30.40
ATOM    136  OE1 GLU A  23      42.946  62.648  80.900  1.00 31.10
ATOM    137  OE2 GLU A  23      43.544  62.118  78.957  1.00 32.35
ATOM    138  N   ALA A  24      38.196  66.359  79.610  1.00 21.49
ATOM    139  CA  ALA A  24      36.751  66.165  79.738  1.00 22.48
ATOM    140  C   ALA A  24      35.973  66.420  78.438  1.00 22.81
ATOM    141  O   ALA A  24      36.325  67.333  77.704  1.00 23.36
ATOM    142  CB  ALA A  24      36.188  67.183  80.734  1.00 21.43
ATOM    143  N   ILE A  25      34.859  65.694  78.228  1.00 23.46
ATOM    144  CA  ILE A  25      33.845  66.149  77.243  1.00 23.60
ATOM    145  C   ILE A  25      33.312  67.535  77.530  1.00 24.71
ATOM    146  O   ILE A  25      32.788  67.809  78.603  1.00 25.03
ATOM    147  CB  ILE A  25      32.684  65.160  77.096  1.00 20.26
ATOM    148  CG1 ILE A  25      33.237  63.749  76.838  1.00 23.14
ATOM    149  CG2 ILE A  25      31.739  65.555  75.954  1.00 21.26
ATOM    150  CD1 ILE A  25      34.298  63.551  75.722  1.00 16.94
ATOM    151  N   ASN A  26      33.485  68.431  76.562  1.00 22.50
ATOM    152  CA  ASN A  26      32.797  69.706  76.751  1.00 22.04
ATOM    153  C   ASN A  26      31.295  69.680  76.533  1.00 22.52
ATOM    154  O   ASN A  26      30.731  70.042  75.509  1.00 22.34
ATOM    155  CB  ASN A  26      33.474  70.744  75.900  1.00 20.15
ATOM    156  CG  ASN A  26      32.982  72.133  76.217  1.00 24.94
ATOM    157  OD1 ASN A  26      31.923  72.459  76.732  1.00 29.46
ATOM    158  ND2 ASN A  26      33.827  73.032  75.809  1.00 25.76
ATOM    159  N   SER A  27      30.627  69.289  77.622  1.00 19.77
ATOM    160  CA  SER A  27      29.166  69.168  77.549  1.00 18.88
ATOM    161  C   SER A  27      28.412  70.423  77.177  1.00 18.74
ATOM    162  O   SER A  27      27.390  70.393  76.517  1.00 21.73
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[Drawing 13]

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ATOM    163  CB  SER A  27      28.606  68.619  78.870  1.00 19.35
ATOM    164  OG  SER A  27      28.967  69.518  79.940  1.00 19.36
ATOM    165  N   LEU A  28      28.961  71.564  77.588  1.00 18.08
ATOM    166  CA  LEU A  28      28.271  72.815  77.262  1.00 20.15
ATOM    167  C   LEU A  28      28.283  73.062  75.761  1.00 23.12
ATOM    168  O   LEU A  28      27.303  73.485  75.165  1.00 22.25
ATOM    169  CB  LEU A  28      28.990  74.042  77.798  1.00 17.00
ATOM    170  CG  LEU A  28      28.159  75.188  78.376  1.00 18.01
ATOM    171  CD1 LEU A  28      26.847  75.547  77.733  1.00 14.28
ATOM    172  CD2 LEU A  28      29.053  76.394  78.592  1.00 16.45
ATOM    173  N   ALA A  29      29.478  72.767  75.193  1.00 23.87
ATOM    174  CA  ALA A  29      29.598  72.827  73.707  1.00 22.62
ATOM    175  C   ALA A  29      28.773  71.847  72.837  1.00 20.86
ATOM    176  O   ALA A  29      28.192  72.239  71.830  1.00 25.89
ATOM    177  CB  ALA A  29      31.065  72.692  73.326  1.00 20.24
ATOM    178  N   LEU A  30      28.733  70.580  73.267  1.00 15.78
ATOM    179  CA  LEU A  30      28.079  69.497  72.519  1.00 18.05
ATOM    180  C   LEU A  30      26.557  69.416  72.559  1.00 22.38
ATOM    181  O   LEU A  30      25.845  69.251  71.566  1.00 23.63
ATOM    182  CB  LEU A  30      28.732  68.194  72.977  1.00 16.47
ATOM    183  CG  LEU A  30      28.234  66.887  72.360  1.00 17.59
ATOM    184  CD1 LEU A  30      28.812  65.706  73.120  1.00 12.95
ATOM    185  CD2 LEU A  30      28.456  66.775  70.850  1.00 13.89
ATOM    186  N   LEU A  31      26.075  69.533  73.812  1.00 22.39
ATOM    187  CA  LEU A  31      24.633  69.430  74.049  1.00 18.84
ATOM    188  C   LEU A  31      23.817  70.624  73.538  1.00 16.30
ATOM    189  O   LEU A  31      24.260  71.763  73.576  1.00 19.93
ATOM    190  CB  LEU A  31      24.381  69.199  75.556  1.00 16.94
ATOM    191  CG  LEU A  31      24.923  67.873  76.095  1.00 17.95
ATOM    192  CD1 LEU A  31      24.177  66.669  75.553  1.00 11.60
ATOM    193  CD2 LEU A  31      24.823  67.878  77.628  1.00 18.77
ATOM    194  N   PRO A  32      22.581  70.333  73.105  1.00 14.80
ATOM    195  CA  PRO A  32      21.589  71.404  72.910  1.00 18.31
ATOM    196  C   PRO A  32      21.228  72.028  74.278  1.00 22.24
ATOM    197  O   PRO A  32      21.453  71.442  75.327  1.00 22.27
ATOM    198  CB  PRO A  32      20.402  70.621  72.348  1.00 15.94
ATOM    199  CG  PRO A  32      20.545  69.184  72.847  1.00 16.93
ATOM    200  CD  PRO A  32      22.038  68.972  72.954  1.00 15.56
ATOM    201  N   PRO A  33      20.657  73.249  74.287  1.00 23.41
ATOM    202  CA  PRO A  33      20.190  73.780  75.586  1.00 20.34
ATOM    203  C   PRO A  33      19.059  72.945  76.084  1.00 19.93
ATOM    204  O   PRO A  33      18.409  72.292  75.285  1.00 18.67
ATOM    205  CB  PRO A  33      19.659  75.158  75.224  1.00 18.52
ATOM    206  CG  PRO A  33      20.267  75.499  73.877  1.00 21.71
ATOM    207  CD  PRO A  33      20.406  74.146  73.177  1.00 21.12
ATOM    208  N   PRO A  34      18.785  72.950  77.411  1.00 19.36
ATOM    209  CA  PRO A  34      17.645  72.138  77.863  1.00 13.70
ATOM    210  C   PRO A  34      16.348  72.759  77.351  1.00 11.77
ATOM    211  O   PRO A  34      16.280  73.937  77.090  1.00 14.58
ATOM    212  CB  PRO A  34      17.760  72.358  79.389  1.00 13.66
ATOM    213  CG  PRO A  34      18.471  73.698  79.571  1.00 14.81
ATOM    214  CD  PRO A  34      19.499  73.679  78.464  1.00 16.49
ATOM    215  N   PRO A  35      15.257  72.007  77.284  1.00 12.52
ATOM    216  CA  PRO A  35      14.011  72.710  76.973  1.00 13.71
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[Drawing 14]

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ATOM    217  C   PRO A  35      13.665  73.842  77.945  1.00 20.26
ATOM    218  O   PRO A  35      13.728  73.715  79.159  1.00 20.52
ATOM    219  CB  PRO A  35      12.997  71.579  76.991  1.00 11.74
ATOM    220  CG  PRO A  35      13.723  70.243  77.051  1.00 12.04
ATOM    221  CD  PRO A  35      15.140  70.581  77.482  1.00 11.57
ATOM    222  N   ALA A  36      13.311  74.962  77.356  1.00 19.25
ATOM    223  CA  ALA A  36      12.919  76.136  78.122  1.00 18.78
ATOM    224  C   ALA A  36      11.457  76.120  78.497  1.00 18.25
ATOM    225  O   ALA A  36      10.582  75.579  77.847  1.00 18.88
ATOM    226  CB  ALA A  36      13.152  77.414  77.304  1.00 17.95
ATOM    227  N   VAL A  37      11.182  76.753  79.609  1.00 18.03
ATOM    228  CA  VAL A  37       9.803  77.005  79.965  1.00 16.78
ATOM    229  C   VAL A  37       9.135  77.993  78.998  1.00 17.18
ATOM    230  O   VAL A  37       9.640  79.048  78.650  1.00 19.89
ATOM    231  CB  VAL A  37       9.740  77.500  81.436  1.00 18.33
ATOM    232  CG1 VAL A  37      10.381  76.501  82.418  1.00 13.83
ATOM    233  CG2 VAL A  37       8.300  77.825  81.832  1.00 14.21
ATOM    234  N   GLY A  38       7.952  77.616  78.561  1.00 18.34
ATOM    235  CA  GLY A  38       7.422  78.249  77.343  1.00 22.06
ATOM    236  C   GLY A  38       7.538  77.398  76.043  1.00 21.25
ATOM    237  O   GLY A  38       6.851  77.623  75.068  1.00 22.09
ATOM    238  N   SER A  39       8.422  76.401  76.060  1.00 21.73
ATOM    239  CA  SER A  39       8.520  75.487  74.905  1.00 20.30
ATOM    240  C   SER A  39       7.604  74.277  74.964  1.00 21.10
ATOM    241  O   SER A  39       7.217  73.736  76.002  1.00 19.55
ATOM    242  CB  SER A  39       9.946  74.998  74.748  1.00 15.45
ATOM    243  OG  SER A  39      10.197  73.967  75.704  1.00 15.38
ATOM    244  N   ILE A  40       7.287  73.796  73.772  1.00 17.17
ATOM    245  CA  ILE A  40       6.618  72.485  73.702  1.00 14.71
ATOM    246  C   ILE A  40       7.475  71.311  74.225  1.00 10.81
ATOM    247  O   ILE A  40       6.998  70.315  74.782  1.00 15.23
ATOM    248  CB  ILE A  40       6.102  72.235  72.219  1.00 15.78
ATOM    249  CG1 ILE A  40       5.162  73.368  71.791  1.00 15.41
ATOM    250  CG2 ILE A  40       5.406  70.863  72.091  1.00 14.54
ATOM    251  CD1 ILE A  40       4.812  73.332  70.307  1.00 18.26
ATOM    252  N   ALA A  41       8.790  71.443  74.040  1.00 10.69
ATOM    253  CA  ALA A  41       9.633  70.373  74.530  1.00 13.79
ATOM    254  C   ALA A  41       9.566  70.300  76.091  1.00 15.36
ATOM    255  O   ALA A  41       9.369  69.245  76.683  1.00 20.02
ATOM    256  CB  ALA A  41      11.046  70.610  74.065  1.00 11.61
ATOM    257  N   PHE A  42       9.547  71.495  76.702  1.00 16.94
ATOM    258  CA  PHE A  42       9.200  71.480  78.151  1.00 15.75
ATOM    259  C   PHE A  42       7.818  70.970  78.533  1.00 16.07
ATOM    260  O   PHE A  42       7.652  70.182  79.448  1.00 19.72
ATOM    261  CB  PHE A  42       9.513  72.819  78.819  1.00 17.93
ATOM    262  CG  PHE A  42       9.380  72.700  80.338  1.00 20.96
ATOM    263  CD1 PHE A  42      10.297  71.904  81.056  1.00 19.46
ATOM    264  CD2 PHE A  42       8.324  73.370  80.997  1.00 20.99
ATOM    265  CE1 PHE A  42      10.148  71.763  82.450  1.00 17.30
ATOM    266  CE2 PHE A  42       8.190  73.248  82.402  1.00 19.79
ATOM    267  CZ  PHE A  42       9.111  72.443  83.100  1.00 13.36
ATOM    268  N   LEU A  43       6.790  71.375  77.765  1.00 19.00
ATOM    269  CA  LEU A  43       5.507  70.643  77.917  1.00 19.22
ATOM    270  C   LEU A  43       5.573  69.103  77.945  1.00 19.39
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[Drawing 15]

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ATOM    271  O   LEU A  43       4.957  68.410  78.749  1.00 17.69
ATOM    272  CB  LEU A  43       4.472  71.003  76.826  1.00 21.60
ATOM    273  CG  LEU A  43       3.213  71.850  77.034  1.00 24.67
ATOM    274  CD1 LEU A  43       2.597  71.800  78.433  1.00 17.68
ATOM    275  CD2 LEU A  43       2.172  71.549  75.953  1.00 21.72
ATOM    276  N   ASN A  44       6.392  68.588  77.023  1.00 19.47
ATOM    277  CA  ASN A  44       6.653  67.176  77.076  1.00 19.82
ATOM    278  C   ASN A  44       7.419  66.619  78.312  1.00 17.44
ATOM    279  O   ASN A  44       7.018  65.604  78.855  1.00 15.52
ATOM    280  CB  ASN A  44       7.259  66.847  75.747  1.00 19.07
ATOM    281  CG  ASN A  44       7.491  65.366  75.643  1.00 22.97
ATOM    282  OD1 ASN A  44       8.605  64.906  75.468  1.00 30.35
ATOM    283  ND2 ASN A  44       6.444  64.588  75.862  1.00 22.13
ATOM    284  N   ASP A  45       8.482  67.324  78.726  1.00 19.71
ATOM    285  CA  ASP A  45       9.175  67.050  80.020  1.00 19.87
ATOM    286  C   ASP A  45       8.192  66.977  81.213  1.00 19.30
ATOM    287  O   ASP A  45       8.103  66.009  81.956  1.00 21.00
ATOM    288  CB  ASP A  45      10.225  68.119  80.273  1.00 13.57
ATOM    289  CG  ASP A  45      11.563  67.769  79.706  1.00 12.64
ATOM    290  OD1 ASP A  45      12.408  68.656  79.625  1.00 15.68
ATOM    291  OD2 ASP A  45      11.823  66.611  79.414  1.00 14.57
ATOM    292  N   GLN A  46       7.347  68.007  81.299  1.00 19.62
ATOM    293  CA  GLN A  46       6.199  67.904  82.220  1.00 19.44
ATOM    294  C   GLN A  46       5.259  66.702  82.166  1.00 22.23
ATOM    295  O   GLN A  46       4.960  66.057  83.175  1.00 21.67
ATOM    296  CB  GLN A  46       5.353  69.153  82.218  1.00 16.35
ATOM    297  CG  GLN A  46       6.282  70.333  82.395  1.00 18.35
ATOM    298  CD  GLN A  46       5.398  71.519  82.591  1.00 26.07
ATOM    299  OE1 GLN A  46       5.334  72.143  83.629  1.00 31.83
ATOM    300  NE2 GLN A  46       4.622  71.823  81.591  1.00 22.82
ATOM    301  N   ALA A  47       4.838  66.364  80.935  1.00 19.12
ATOM    302  CA  ALA A  47       3.979  65.187  80.813  1.00 17.83
ATOM    303  C   ALA A  47       4.661  63.871  81.172  1.00 15.90
ATOM    304  O   ALA A  47       4.065  62.940  81.701  1.00 18.55
ATOM    305  CB  ALA A  47       3.441  65.066  79.367  1.00 17.11
ATOM    306  N   MET A  48       5.970  63.818  80.841  1.00 18.16
ATOM    307  CA  MET A  48       6.799  62.644  81.235  1.00 19.52
ATOM    308  C   MET A  48       7.012  62.460  82.765  1.00 21.38
ATOM    309  O   MET A  48       6.996  61.358  83.316  1.00 20.83
ATOM    310  CB  MET A  48       8.173  62.667  80.539  1.00 21.42
ATOM    311  CG  MET A  48       8.150  62.603  78.984  1.00 29.81
ATOM    312  SD  MET A  48       7.330  61.126  78.308  1.00 36.20
ATOM    313  CE  MET A  48       5.582  61.633  78.280  1.00 33.60
ATOM    314  N   TYR A  49       7.139  63.655  83.414  1.00 21.32
ATOM    315  CA  TYR A  49       7.066  63.807  84.885  1.00 21.30
ATOM    316  C   TYR A  49       5.773  63.244  85.515  1.00 22.58
ATOM    317  O   TYR A  49       5.797  62.383  86.390  1.00 24.04
ATOM    318  CB  TYR A  49       7.304  65.282  85.217  1.00 20.61
ATOM    319  CG  TYR A  49       7.034  65.494  86.692  1.00 23.57
ATOM    320  CD1 TYR A  49       5.755  65.931  87.109  1.00 23.57
ATOM    321  CD2 TYR A  49       8.080  65.194  87.574  1.00 21.83
ATOM    322  CE1 TYR A  49       5.524  66.097  88.481  1.00 26.09
ATOM    323  CE2 TYR A  49       7.844  65.349  88.943  1.00 23.18
ATOM    324  CZ  TYR A  49       6.591  65.842  89.377  1.00 26.31
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[Drawing 16]

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ATOM    325  OH  TYR A  49       6.444  66.124  90.726  1.00 29.46
ATOM    326  N   GLU A  50       4.639  63.731  84.994  1.00 22.09
ATOM    327  CA  GLU A  50       3.336  63.234  85.472  1.00 21.48
ATOM    328  C   GLU A  50       3.052  61.776  85.230  1.00 23.20
ATOM    329  O   GLU A  50       2.548  61.050  86.081  1.00 24.23
ATOM    330  CB  GLU A  50       2.190  64.023  84.862  1.00 21.88
ATOM    331  CG  GLU A  50       2.304  65.537  84.986  1.00 21.13
ATOM    332  CD  GLU A  50       2.054  65.976  86.417  1.00 25.41
ATOM    333  OE1 GLU A  50       1.887  65.138  87.287  1.00 24.65
ATOM    334  OE2 GLU A  50       2.004  67.162  86.679  1.00 25.05
ATOM    335  N   GLN A  51       3.479  61.343  84.032  1.00 23.02
ATOM    336  CA  GLN A  51       3.427  59.907  83.812  1.00 24.72
ATOM    337  C   GLN A  51       4.275  59.006  84.728  1.00 26.23
ATOM    338  O   GLN A  51       3.804  57.996  85.253  1.00 25.10
ATOM    339  CB  GLN A  51       3.680  59.545  82.355  1.00 24.41
ATOM    340  CG  GLN A  51       3.461  58.028  82.141  1.00 38.05
ATOM    341  CD  GLN A  51       2.115  57.435  82.657  1.00 53.15
ATOM    342  OE1 GLN A  51       1.093  58.076  82.867  1.00 61.03
ATOM    343  NE2 GLN A  51       2.098  56.123  82.834  1.00 55.24
ATOM    344  N   GLY A  52       5.556  59.414  84.922  1.00 26.76
ATOM    345  CA  GLY A  52       6.400  58.689  85.876  1.00 26.91
ATOM    346  C   GLY A  52       5.793  58.681  87.286  1.00 25.60
ATOM    347  O   GLY A  52       5.666  57.699  87.997  1.00 24.37
ATOM    348  N   ARG A  53       5.321  59.874  87.621  1.00 28.09
ATOM    349  CA  ARG A  53       4.527  60.032  88.834  1.00 29.75
ATOM    350  C   ARG A  53       3.384  59.049  89.067  1.00 32.01
ATOM    351  O   ARG A  53       3.284  58.437  90.115  1.00 34.69
ATOM    352  CB  ARG A  53       4.128  61.494  88.965  1.00 30.41
ATOM    353  CG  ARG A  53       3.857  61.919  90.389  1.00 29.15
ATOM    354  CD  ARG A  53       3.519  63.393  90.461  1.00 29.38
ATOM    355  NE  ARG A  53       2.385  63.740  89.609  1.00 31.35
ATOM    356  CZ  ARG A  53       1.088  63.593  89.886  1.00 32.29
ATOM    357  NH1 ARG A  53       0.187  64.144  89.125  1.00 32.06
ATOM    358  NH2 ARG A  53       0.661  62.938  90.931  1.00 32.66
ATOM    359  N   LEU A  54       2.575  58.839  88.033  1.00 32.47
ATOM    360  CA  LEU A  54       1.588  57.735  88.076  1.00 31.67
ATOM    361  C   LEU A  54       2.114  56.273  88.119  1.00 33.30
ATOM    362  O   LEU A  54       1.452  55.329  88.568  1.00 35.90
ATOM    363  CB  LEU A  54       0.603  57.880  86.901  1.00 33.52
ATOM    364  CG  LEU A  54      -0.599  58.822  87.055  1.00 32.85
ATOM    365  CD1 LEU A  54      -1.298  59.020  85.709  1.00 30.53
ATOM    366  CD2 LEU A  54      -0.286  60.130  87.777  1.00 36.56
ATOM    367  N   LEU A  55       3.374  56.117  87.657  1.00 31.95
ATOM    368  CA  LEU A  55       4.016  54.784  87.735  1.00 32.70
ATOM    369  C   LEU A  55       4.577  54.392  89.091  1.00 33.98
ATOM    370  O   LEU A  55       4.842  53.224  89.386  1.00 32.73
ATOM    371  CB  LEU A  55       5.194  54.646  86.790  1.00 31.04
ATOM    372  CG  LEU A  55       4.832  54.514  85.343  1.00 28.60
ATOM    373  CD1 LEU A  55       3.960  53.287  85.101  1.00 27.88
ATOM    374  CD2 LEU A  55       6.118  54.471  84.539  1.00 28.33
ATOM    375  N   ARG A  56       4.732  55.458  89.911  1.00 36.20
ATOM    376  CA  ARG A  56       5.257  55.308  91.281  1.00 37.04
ATOM    377  C   ARG A  56       4.616  54.240  92.164  1.00 40.28
ATOM    378  O   ARG A  56       5.260  53.518  92.907  1.00 41.22
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[Drawing 17]

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ATOM    379  CB  ARG A  56       5.249  56.643  91.993  1.00 32.58
ATOM    380  CG  ARG A  56       6.368  57.502  91.476  1.00 22.04
ATOM    381  CD  ARG A  56       6.142  58.874  92.049  1.00 21.74
ATOM    382  NE  ARG A  56       7.073  59.804  91.447  1.00 23.56
ATOM    383  CZ  ARG A  56       7.062  61.074  91.750  1.00 25.56
ATOM    384  NH1 ARG A  56       6.401  61.444  92.790  1.00 30.94
ATOM    385  NH2 ARG A  56       7.688  61.979  91.035  1.00 22.33
ATOM    386  N   ASN A  57       3.306  54.120  91.997  1.00 44.42
ATOM    387  CA  ASN A  57       2.602  53.027  92.680  1.00 48.62
ATOM    388  C   ASN A  57       2.786  51.585  92.169  1.00 47.46
ATOM    389  O   ASN A  57       2.316  50.630  92.759  1.00 51.15
ATOM    390  CB  ASN A  57       1.124  53.435  92.726  1.00 59.30
ATOM    391  CG  ASN A  57       0.389  53.137  94.049  1.00 68.98
ATOM    392  OD1 ASN A  57      -0.829  53.335  94.164  1.00 75.09
ATOM    393  ND2 ASN A  57       1.140  52.692  95.058  1.00 71.68
ATOM    394  N   THR A  58       3.461  51.442  91.036  1.00 42.68
ATOM    395  CA  THR A  58       3.555  50.086  90.475  1.00 36.64
ATOM    396  C   THR A  58       4.821  49.318  90.871  1.00 33.64
ATOM    397  O   THR A  58       5.721  49.876  91.477  1.00 31.69
ATOM    398  CB  THR A  58       3.492  50.189  88.948  1.00 36.81
ATOM    399  OG1 THR A  58       4.774  50.576  88.447  1.00 37.64
ATOM    400  CG2 THR A  58       2.432  51.203  88.507  1.00 35.36
ATOM    401  N   GLU A  59       4.937  48.068  90.409  1.00 33.08
ATOM    402  CA  GLU A  59       6.238  47.410  90.581  1.00 34.80
ATOM    403  C   GLU A  59       7.487  48.104  89.944  1.00 33.45
ATOM    404  O   GLU A  59       8.607  48.153  90.463  1.00 34.28
ATOM    405  CB  GLU A  59       6.067  45.933  90.191  1.00 43.43
ATOM    406  CG  GLU A  59       7.242  45.007  90.614  1.00 59.74
ATOM    407  CD  GLU A  59       7.519  44.933  92.159  1.00 69.61
ATOM    408  OE1 GLU A  59       6.582  45.064  92.960  1.00 74.78
ATOM    409  OE2 GLU A  59       8.686  44.751  92.589  1.00 74.97
ATOM    410  N   ARG A  60       7.229  48.734  88.768  1.00 27.61
ATOM    411  CA  ARG A  60       8.251  49.599  88.158  1.00 25.02
ATOM    412  C   ARG A  60       8.614  50.851  88.958  1.00 22.94
ATOM    413  O   ARG A  60       9.772  51.257  89.002  1.00 24.63
ATOM    414  CB  ARG A  60       7.874  49.966  86.690  1.00 26.16
ATOM    415  CG  ARG A  60       8.877  50.860  85.900  1.00 24.47
ATOM    416  CD  ARG A  60      10.268  50.249  85.758  1.00 23.96
ATOM    417  NE  ARG A  60      11.285  51.161  85.217  1.00 25.64
ATOM    418  CZ  ARG A  60      12.214  51.778  85.945  1.00 24.77
ATOM    419  NH1 ARG A  60      12.159  51.805  87.261  1.00 24.78
ATOM    420  NH2 ARG A  60      13.227  52.294  85.325  1.00 19.79
ATOM    421  N   GLY A  61       7.562  51.411  89.587  1.00 21.94
ATOM    422  CA  GLY A  61       7.623  52.443  90.620  1.00 22.33
ATOM    423  C   GLY A  61       8.468  52.051  91.824  1.00 24.44
ATOM    424  O   GLY A  61       9.350  52.773  92.253  1.00 25.22
ATOM    425  N   LYS A  62       8.248  50.821  92.307  1.00 26.95
ATOM    426  CA  LYS A  62       9.102  50.251  93.350  1.00 26.24
ATOM    427  C   LYS A  62      10.590  50.158  93.045  1.00 24.89
ATOM    428  O   LYS A  62      11.443  50.668  93.756  1.00 23.23
ATOM    429  CB  LYS A  62       8.519  48.900  93.723  1.00 29.90
ATOM    430  CG  LYS A  62       9.379  48.296  94.835  1.00 38.76
ATOM    431  CD  LYS A  62       8.847  46.904  95.222  1.00 47.47
ATOM    432  CE  LYS A  62       9.944  45.971  95.773  1.00 53.72
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[Drawing 18]

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ATOM    433  NZ  LYS A  62      10.167  44.857  94.832  1.00 60.22
ATOM    434  N   LEU A  63      10.866  49.560  91.882  1.00 24.67
ATOM    435  CA  LEU A  63      12.239  49.634  91.346  1.00 23.65
ATOM    436  C   LEU A  63      12.805  51.043  91.186  1.00 22.74
ATOM    437  O   LEU A  63      13.927  51.359  91.517  1.00 25.19
ATOM    438  CB  LEU A  63      12.232  48.973  89.981  1.00 27.11
ATOM    439  CG  LEU A  63      13.477  48.298  89.403  1.00 31.83
ATOM    440  CD1 LEU A  63      14.808  48.658  90.077  1.00 33.84
ATOM    441  CD2 LEU A  63      13.440  48.410  87.874  1.00 26.95
ATOM    442  N   ALA A  64      11.979  51.940  90.642  1.00 21.62
ATOM    443  CA  ALA A  64      12.492  53.308  90.539  1.00 20.51
ATOM    444  C   ALA A  64      12.862  53.971  91.863  1.00 21.79
ATOM    445  O   ALA A  64      13.890  54.636  91.984  1.00 21.21
ATOM    446  CB  ALA A  64      11.456  54.196  89.862  1.00 19.01
ATOM    447  N   ALA A  65      11.984  53.747  92.870  1.00 23.25
ATOM    448  CA  ALA A  65      12.374  54.212  94.235  1.00 24.88
ATOM    449  C   ALA A  65      13.684  53.619  94.784  1.00 22.62
ATOM    450  O   ALA A  65      14.551  54.328  95.268  1.00 22.95
ATOM    451  CB  ALA A  65      11.249  54.013  95.265  1.00 25.38
ATOM    452  N   GLU A  66      13.848  52.295  94.572  1.00 23.38
ATOM    453  CA  GLU A  66      15.116  51.632  94.878  1.00 23.41
ATOM    454  C   GLU A  66      16.332  52.188  94.152  1.00 26.06
ATOM    455  O   GLU A  66      17.321  52.604  94.744  1.00 25.12
ATOM    456  CB  GLU A  66      14.968  50.136  94.665  1.00 25.85
ATOM    457  CG  GLU A  66      13.818  49.616  95.533  1.00 31.94
ATOM    458  CD  GLU A  66      13.546  48.142  95.293  1.00 37.59
ATOM    459  OE1 GLU A  66      13.147  47.430  96.220  1.00 40.33
ATOM    460  OE2 GLU A  66      13.721  47.673  94.176  1.00 40.79
ATOM    461  N   ASP A  67      16.204  52.276  92.817  1.00 24.01
ATOM    462  CA  ASP A  67      17.222  52.928  91.986  1.00 19.72
ATOM    463  C   ASP A  67      17.549  54.333  92.402  1.00 16.72
ATOM    464  O   ASP A  67      18.694  54.767  92.414  1.00 18.91
ATOM    465  CB  ASP A  67      16.787  52.944  90.495  1.00 21.68
ATOM    466  CG  ASP A  67      16.824  51.580  89.801  1.00 25.22
ATOM    467  OD1 ASP A  67      17.340  50.629  90.370  1.00 23.32
ATOM    468  OD2 ASP A  67      16.349  51.434  88.666  1.00 26.83
ATOM    469  N   ALA A  68      16.485  55.059  92.773  1.00 16.48
ATOM    470  CA  ALA A  68      16.685  56.425  93.250  1.00 19.28
ATOM    471  C   ALA A  68      17.489  56.510  94.569  1.00 20.86
ATOM    472  O   ALA A  68      18.165  57.494  94.837  1.00 22.07
ATOM    473  CB  ALA A  68      15.330  57.134  93.419  1.00 19.81
ATOM    474  N   ASN A  69      17.472  55.371  95.299  1.00 23.11
ATOM    475  CA  ASN A  69      18.330  55.262  96.514  1.00 27.41
ATOM    476  C   ASN A  69      19.816  55.042  96.273  1.00 29.49
ATOM    477  O   ASN A  69      20.646  55.304  97.140  1.00 28.64
ATOM    478  CB  ASN A  69      17.933  54.145  97.466  1.00 24.19
ATOM    479  CG  ASN A  69      16.632  54.425  98.142  1.00 25.79
ATOM    480  OD1 ASN A  69      16.298  55.549  98.445  1.00 26.84
ATOM    481  ND2 ASN A  69      15.894  53.359  98.410  1.00 30.16
ATOM    482  N   LEU A  70      20.104  54.574  95.034  1.00 25.30
ATOM    483  CA  LEU A  70      21.514  54.442  94.627  1.00 23.20
ATOM    484  C   LEU A  70      22.329  55.691  94.640  1.00 21.41
ATOM    485  O   LEU A  70      22.013  56.696  94.028  1.00 23.50
ATOM    486  CB  LEU A  70      21.672  53.890  93.225  1.00 22.19
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[Drawing 19]

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ATOM    487  CG  LEU A  70      21.078  52.512  93.095  1.00 22.16
ATOM    488  CD1 LEU A  70      21.830  51.459  93.896  1.00 20.70
ATOM    489  CD2 LEU A  70      21.016  52.154  91.624  1.00 23.47
ATOM    490  N   SER A  71      23.450  55.563  95.304  1.00 21.73
ATOM    491  CA  SER A  71      24.527  56.515  95.119  1.00 22.25
ATOM    492  C   SER A  71      25.355  56.171  93.888  1.00 20.52
ATOM    493  O   SER A  71      25.269  55.081  93.357  1.00 23.70
ATOM    494  CB  SER A  71      25.453  56.521  96.349  1.00 22.74
ATOM    495  OG  SER A  71      26.232  55.303  96.432  1.00 28.68
ATOM    496  N   SER A  72      26.220  57.079  93.445  1.00 20.02
ATOM    497  CA  SER A  72      27.096  56.747  92.294  1.00 20.88
ATOM    498  C   SER A  72      27.860  55.479  92.410  1.00 22.52
ATOM    499  O   SER A  72      27.979  54.663  91.518  1.00 21.65
ATOM    500  CB  SER A  72      28.113  57.834  92.083  1.00 19.05
ATOM    501  OG  SER A  72      27.352  58.966  91.735  1.00 22.00
ATOM    502  N   GLY A  73      28.336  55.318  93.640  1.00 20.71
ATOM    503  CA  GLY A  73      28.979  54.068  94.006  1.00 16.81
ATOM    504  C   GLY A  73      28.146  52.783  93.939  1.00 15.97
ATOM    505  O   GLY A  73      28.697  51.705  93.753  1.00 20.02
ATOM    506  N   GLY A  74      26.818  52.915  94.046  1.00 16.07
ATOM    507  CA  GLY A  74      26.090  51.649  93.967  1.00 18.17
ATOM    508  C   GLY A  74      25.671  51.260  92.526  1.00 21.98
ATOM    509  O   GLY A  74      25.202  50.164  92.238  1.00 21.28
ATOM    510  N   VAL A  75      25.887  52.210  91.567  1.00 22.48
ATOM    511  CA  VAL A  75      25.521  51.777  90.174  1.00 22.71
ATOM    512  C   VAL A  75      26.174  50.493  89.628  1.00 18.50
ATOM    513  O   VAL A  75      25.497  49.573  89.210  1.00 20.32
ATOM    514  CB  VAL A  75      25.820  52.946  89.218  1.00 23.52
ATOM    515  CG1 VAL A  75      25.719  52.707  87.712  1.00 21.49
ATOM    516  CG2 VAL A  75      25.153  54.265  89.560  1.00 17.54
ATOM    517  N   ALA A  76      27.517  50.394  89.738  1.00 21.05
ATOM    518  CA  ALA A  76      28.149  49.125  89.372  1.00 20.51
ATOM    519  C   ALA A  76      27.414  47.875  89.826  1.00 24.64
ATOM    520  O   ALA A  76      27.033  47.028  89.015  1.00 24.90
ATOM    521  CB  ALA A  76      29.612  49.071  89.810  1.00 19.14
ATOM    522  N   ASN A  77      27.131  47.820  91.145  1.00 20.97
ATOM    523  CA  ASN A  77      26.463  46.601  91.622  1.00 17.62
ATOM    524  C   ASN A  77      25.019  46.464  91.205  1.00 16.57
ATOM    525  O   ASN A  77      24.536  45.350  91.024  1.00 19.24
ATOM    526  CB  ASN A  77      26.615  46.509  93.137  1.00 23.52
ATOM    527  CG  ASN A  77      25.817  45.362  93.677  1.00 21.57
ATOM    528  OD1 ASN A  77      24.672  45.508  94.079  1.00 26.66
ATOM    529  ND2 ASN A  77      26.435  44.202  93.627  1.00 24.64
ATOM    530  N   ALA A  78      24.377  47.638  91.017  1.00 17.23
ATOM    531  CA  ALA A  78      23.060  47.710  90.339  1.00 18.62
ATOM    532  C   ALA A  78      22.874  47.025  88.941  1.00 19.92
ATOM    533  O   ALA A  78      21.767  46.705  88.517  1.00 21.04
ATOM    534  CB  ALA A  78      22.636  49.160  90.208  1.00 13.62
ATOM    535  N   PHE A  79      24.025  46.748  88.292  1.00 19.22
ATOM    536  CA  PHE A  79      24.019  45.921  87.070  1.00 20.71
ATOM    537  C   PHE A  79      24.117  44.420  87.238  1.00 23.30
ATOM    538  O   PHE A  79      24.161  43.662  86.273  1.00 23.64
ATOM    539  CB  PHE A  79      25.116  46.352  86.082  1.00 18.00
ATOM    540  CG  PHE A  79      24.821  47.683  85.382  1.00 19.59
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[Drawing 20]

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ATOM    541  CD1 PHE A  79      25.181  48.903  85.984  1.00 17.05
ATOM    542  CD2 PHE A  79      24.214  47.687  84.104  1.00 18.56
ATOM    543  CE1 PHE A  79      25.026  50.119  85.298  1.00 19.06
ATOM    544  CE2 PHE A  79      24.040  48.906  83.419  1.00 15.97
ATOM    545  CZ  PHE A  79      24.500  50.104  83.989  1.00 17.87
ATOM    546  N   SER A  80      24.150  43.947  88.488  1.00 19.74
ATOM    547  CA  SER A  80      24.500  42.511  88.632  1.00 17.29
ATOM    548  C   SER A  80      23.601  41.501  87.944  1.00 18.57
ATOM    549  O   SER A  80      23.977  40.494  87.339  1.00 22.38
ATOM    550  CB  SER A  80      24.608  42.106  90.125  1.00 15.17
ATOM    551  OG  SER A  80      25.646  42.920  90.700  1.00 17.32
ATOM    552  N   GLY A  81      22.309  41.852  88.041  1.00 19.68
ATOM    553  CA  GLY A  81      21.271  41.005  87.413  1.00 22.50
ATOM    554  C   GLY A  81      21.293  40.977  85.855  1.00 24.85
ATOM    555  O   GLY A  81      21.318  39.939  85.211  1.00 23.46
ATOM    556  N   ALA A  82      21.380  42.197  85.279  1.00 24.60
ATOM    557  CA  ALA A  82      21.686  42.339  83.855  1.00 24.50
ATOM    558  C   ALA A  82      22.985  41.643  83.417  1.00 25.22
ATOM    559  O   ALA A  82      23.000  40.873  82.468  1.00 23.42
ATOM    560  CB  ALA A  82      21.649  43.819  83.470  1.00 20.35
ATOM    561  N   PHE A  83      24.050  41.874  84.197  1.00 23.76
ATOM    562  CA  PHE A  83      25.319  41.242  83.894  1.00 21.76
ATOM    563  C   PHE A  83      25.325  39.726  83.974  1.00 22.85
ATOM    564  O   PHE A  83      26.090  39.052  83.322  1.00 25.72
ATOM    565  CB  PHE A  83      26.349  41.867  84.792  1.00 20.01
ATOM    566  CG  PHE A  83      27.770  41.527  84.394  1.00 20.95
ATOM    567  CD1 PHE A  83      28.486  40.587  85.152  1.00 16.94
ATOM    568  CD2 PHE A  83      28.391  42.208  83.307  1.00 23.09
ATOM    569  CE1 PHE A  83      29.841  40.355  84.843  1.00 18.79
ATOM    570  CE2 PHE A  83      29.751  41.991  83.001  1.00 20.95
ATOM    571  CZ  PHE A  83      30.474  41.069  83.795  1.00 21.43
ATOM    572  N   GLY A  84      24.409  39.187  84.768  1.00 22.31
ATOM    573  CA  GLY A  84      24.478  37.740  84.865  1.00 24.39
ATOM    574  C   GLY A  84      25.199  37.163  86.093  1.00 29.49
ATOM    575  O   GLY A  84      25.158  35.963  86.362  1.00 32.27
ATOM    576  N   SER A  85      25.873  38.058  86.843  1.00 30.33
ATOM    577  CA  SER A  85      26.685  37.625  88.001  1.00 28.46
ATOM    578  C   SER A  85      27.047  38.788  88.936  1.00 29.06
ATOM    579  O   SER A  85      26.915  39.945  88.556  1.00 29.00
ATOM    580  CB  SER A  85      27.915  36.861  87.536  1.00 24.04
ATOM    581  OG  SER A  85      28.903  37.746  87.028  1.00 28.11
ATOM    582  N   PRO A  86      27.436  38.518  90.216  1.00 29.45
ATOM    583  CA  PRO A  86      27.599  39.650  91.122  1.00 26.27
ATOM    584  C   PRO A  86      28.721  40.513  90.733  1.00 22.75
ATOM    585  O   PRO A  86      29.830  40.064  90.530  1.00 22.43
ATOM    586  CB  PRO A  86      27.873  39.029  92.493  1.00 27.52
ATOM    587  CG  PRO A  86      27.284  37.627  92.399  1.00 27.61
ATOM    588  CD  PRO A  86      27.591  37.258  90.945  1.00 31.08
ATOM    589  N   ILE A  87      28.350  41.776  90.659  1.00 22.99
ATOM    590  CA  ILE A  87      29.363  42.816  90.469  1.00 24.80
ATOM    591  C   ILE A  87      29.642  43.494  91.811  1.00 25.76
ATOM    592  O   ILE A  87      28.956  44.424  92.220  1.00 24.68
ATOM    593  CB  ILE A  87      28.908  43.860  89.427  1.00 24.26
ATOM    594  CG1 ILE A  87      28.626  43.165  88.076  1.00 23.29
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[Drawing 21]

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ATOM    595  CG2 ILE A  87      29.997  44.923  89.306  1.00 23.62
ATOM    596  CD1 ILE A  87      27.925  44.100  87.092  1.00 21.95
ATOM    597  N   THR A  88      30.655  42.914  92.481  1.00 25.86
ATOM    598  CA  THR A  88      30.925  43.247  93.903  1.00 25.48
ATOM    599  C   THR A  88      32.418  43.249  94.166  1.00 25.25
ATOM    600  O   THR A  88      33.131  42.561  93.445  1.00 24.26
ATOM    601  CB  THR A  88      30.332  42.211  94.859  1.00 22.31
ATOM    602  OG1 THR A  88      31.102  41.029  94.702  1.00 25.42
ATOM    603  CG2 THR A  88      28.833  41.943  94.710  1.00 19.45
ATOM    604  N   GLU A  89      32.891  43.970  95.204  1.00 26.63
ATOM    605  CA  GLU A  89      34.322  43.845  95.577  1.00 25.29
ATOM    606  C   GLU A  89      34.810  42.429  95.889  1.00 25.30
ATOM    607  O   GLU A  89      35.924  41.999  95.611  1.00 26.30
ATOM    608  CB  GLU A  89      34.652  44.773  96.741  1.00 25.64
ATOM    609  CG  GLU A  89      34.334  46.193  96.340  1.00 26.52
ATOM    610  CD  GLU A  89      34.551  47.228  97.414  1.00 29.70
ATOM    611  OE1 GLU A  89      35.136  48.245  97.123  1.00 33.05
ATOM    612  OE2 GLU A  89      34.138  47.077  98.540  1.00 27.59
ATOM    613  N   LYS A  90      33.860  41.697  96.459  1.00 26.25
ATOM    614  CA  LYS A  90      34.095  40.310  96.883  1.00 28.81
ATOM    615  C   LYS A  90      34.285  39.313  95.780  1.00 28.19
ATOM    616  O   LYS A  90      35.206  38.518  95.773  1.00 30.49
ATOM    617  CB  LYS A  90      32.889  39.869  97.672  1.00 31.00
ATOM    618  CG  LYS A  90      32.956  38.478  98.228  1.00 37.00
ATOM    619  CD  LYS A  90      31.536  38.026  98.583  1.00 43.53
ATOM    620  CE  LYS A  90      31.386  36.504  98.712  1.00 50.17
ATOM    621  NZ  LYS A  90      32.257  35.875  97.701  1.00 60.80
ATOM    622  N   ASP A  91      33.324  39.416  94.870  1.00 28.68
ATOM    623  CA  ASP A  91      33.271  38.504  93.738  1.00 29.34
ATOM    624  C   ASP A  91      33.911  38.947  92.420  1.00 29.37
ATOM    625  O   ASP A  91      34.429  38.173  91.635  1.00 31.54
ATOM    626  CB  ASP A  91      31.827  38.162  93.438  1.00 30.91
ATOM    627  CG  ASP A  91      31.087  37.722  94.674  1.00 31.69
ATOM    628  OD1 ASP A  91      31.395  36.657  95.212  1.00 35.66
ATOM    629  OD2 ASP A  91      30.186  38.438  95.088  1.00 29.63
ATOM    630  N   ALA A  92      33.830  40.240  92.165  1.00 28.62
ATOM    631  CA  ALA A  92      34.443  40.713  90.919  1.00 26.75
ATOM    632  C   ALA A  92      35.255  41.981  91.131  1.00 26.94
ATOM    633  O   ALA A  92      34.937  43.081  90.690  1.00 26.94
ATOM    634  CB  ALA A  92      33.390  40.934  89.817  1.00 23.93
ATOM    635  N   PRO A  93      36.336  41.826  91.930  1.00 27.16
ATOM    636  CA  PRO A  93      37.151  43.015  92.274  1.00 25.78
ATOM    637  C   PRO A  93      37.832  43.865  91.160  1.00 24.62
ATOM    638  O   PRO A  93      37.844  45.098  91.178  1.00 22.75
ATOM    639  CB  PRO A  93      38.120  42.411  93.292  1.00 25.31
ATOM    640  CG  PRO A  93      38.219  40.926  92.945  1.00 23.54
ATOM    641  CD  PRO A  93      36.817  40.578  92.534  1.00 25.29
ATOM    642  N   ALA A  94      38.409  43.174  90.170  1.00 25.16
ATOM    643  CA  ALA A  94      38.954  43.886  89.005  1.00 23.77
ATOM    644  C   ALA A  94      37.923  44.715  88.249  1.00 17.72
ATOM    645  O   ALA A  94      38.116  45.897  88.005  1.00 19.91
ATOM    646  CB  ALA A  94      39.676  42.931  88.058  1.00 21.52
ATOM    647  N   LEU A  95      36.787  44.081  88.026  1.00 19.68
ATOM    648  CA  LEU A  95      35.577  44.770  87.539  1.00 20.23
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[Drawing 22]

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ATOM    649  C   LEU A  95      35.001  45.902  88.385  1.00 21.85
ATOM    650  O   LEU A  95      34.809  47.026  87.943  1.00 21.66
ATOM    651  CB  LEU A  95      34.466  43.755  87.271  1.00 20.70
ATOM    652  CG  LEU A  95      33.250  44.285  86.467  1.00 21.32
ATOM    653  CD1 LEU A  95      32.299  43.149  86.063  1.00 20.10
ATOM    654  CD2 LEU A  95      33.698  45.111  85.240  1.00 20.93
ATOM    655  N   HIS A  96      34.755  45.606  89.667  1.00 21.70
ATOM    656  CA  HIS A  96      34.313  46.691  90.543  1.00 17.84
ATOM    657  C   HIS A  96      35.214  47.924  90.580  1.00 17.76
ATOM    658  O   HIS A  96      34.767  49.069  90.496  1.00 19.44
ATOM    659  CB  HIS A  96      34.042  46.116  91.937  1.00 20.62
ATOM    660  CG  HIS A  96      32.934  46.893  92.613  1.00 21.96
ATOM    661  ND1 HIS A  96      33.021  48.094  93.229  1.00 21.34
ATOM    662  CD2 HIS A  96      31.614  46.470  92.680  1.00 24.62
ATOM    663  CE1 HIS A  96      31.790  48.442  93.690  1.00 18.16
ATOM    664  NE2 HIS A  96      30.923  47.437  93.338  1.00 23.59
ATOM    665  N   LYS A  97      36.539  47.639  90.629  1.00 18.11
ATOM    666  CA  LYS A  97      37.544  48.713  90.581  1.00 18.50
ATOM    667  C   LYS A  97      37.519  49.564  89.317  1.00 20.80
ATOM    668  O   LYS A  97      37.452  50.781  89.373  1.00 21.08
ATOM    669  CB  LYS A  97      38.924  48.085  90.766  1.00 17.26
ATOM    670  CG  LYS A  97      40.125  49.014  90.594  1.00 21.24
ATOM    671  CD  LYS A  97      40.283  50.213  91.525  1.00 30.10
ATOM    672  CE  LYS A  97      41.482  51.160  91.164  1.00 33.55
ATOM    673  NZ  LYS A  97      41.557  52.449  91.915  1.00 29.55
ATOM    674  N   LEU A  98      37.532  48.857  88.155  1.00 22.36
ATOM    675  CA  LEU A  98      37.291  49.555  86.851  1.00 22.04
ATOM    676  C   LEU A  98      36.128  50.581  86.806  1.00 17.59
ATOM    677  O   LEU A  98      36.223  51.763  86.522  1.00 18.13
ATOM    678  CB  LEU A  98      37.025  48.477  85.780  1.00 21.44
ATOM    679  CG  LEU A  98      36.766  49.042  84.375  1.00 20.93
ATOM    680  CD1 LEU A  98      36.265  47.902  83.493  1.00 22.92
ATOM    681  CD2 LEU A  98      37.963  49.801  83.811  1.00 18.27
ATOM    682  N   LEU A  99      34.977  50.024  87.188  1.00 19.16
ATOM    683  CA  LEU A  99      33.753  50.802  87.186  1.00 18.37
ATOM    684  C   LEU A  99      33.644  51.930  88.169  1.00 20.61
ATOM    685  O   LEU A  99      33.068  52.964  87.883  1.00 18.31
ATOM    686  CB  LEU A  99      32.545  49.874  87.263  1.00 18.90
ATOM    687  CG  LEU A  99      32.428  48.860  86.191  1.00 20.87
ATOM    688  CD1 LEU A  99      32.464  49.503  84.841  1.00 14.73
ATOM    689  CD2 LEU A  99      31.190  48.099  86.497  1.00 19.82
ATOM    690  N   THR A 100      34.252  51.692  89.359  1.00 22.03
ATOM    691  CA  THR A 100      34.357  52.777  90.360  1.00 19.41
ATOM    692  C   THR A 100      35.259  53.938  89.957  1.00 16.72
ATOM    693  O   THR A 100      34.984  55.118  90.136  1.00 16.55
ATOM    694  CB  THR A 100      34.889  52.174  91.674  1.00 19.50
ATOM    695  OG1 THR A 100      34.030  51.113  92.091  1.00 19.99
ATOM    696  CG2 THR A 100      35.092  53.221  92.758  1.00 21.12
ATOM    697  N   ASN A 101      36.397  53.537  89.358  1.00 20.56
ATOM    698  CA  ASN A 101      37.415  54.515  88.998  1.00 19.91
ATOM    699  C   ASN A 101      37.022  55.479  87.873  1.00 21.88
ATOM    700  O   ASN A 101      37.610  56.546  87.711  1.00 22.38
ATOM    701  CB  ASN A 101      38.692  53.763  88.716  1.00 20.98
ATOM    702  CG  ASN A 101      39.950  54.556  89.041  1.00 22.36
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[Drawing 23]

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ATOM    703  OD1 ASN A 101      40.938  53.978  89.459  1.00 30.43
ATOM    704  ND2 ASN A 101      39.964  55.868  88.889  1.00 23.45
ATOM    705  N   MET A 102      35.952  55.090  87.154  1.00 21.94
ATOM    706  CA  MET A 102      35.407  55.984  86.103  1.00 22.28
ATOM    707  C   MET A 102      34.142  56.755  86.455  1.00 22.36
ATOM    708  O   MET A 102      33.571  57.482  85.638  1.00 23.37
ATOM    709  CB  MET A 102      35.162  55.213  84.781  1.00 19.03
ATOM    710  CG  MET A 102      34.239  54.001  84.972  1.00 18.05
ATOM    711  SD  MET A 102      33.744  53.082  83.481  1.00 20.07
ATOM    712  CE  MET A 102      32.429  54.165  83.010  1.00 16.04
ATOM    713  N   ILE A 103      33.681  56.555  87.724  1.00 21.00
ATOM    714  CA  ILE A 103      32.441  57.221  88.180  1.00 18.78
ATOM    715  C   ILE A 103      32.371  58.697  87.833  1.00 17.38
ATOM    716  O   ILE A 103      31.413  59.152  87.245  1.00 18.08
ATOM    717  CB  ILE A 103      32.174  57.025  89.732  1.00 16.18
ATOM    718  CG1 ILE A 103      31.696  55.603  90.031  1.00 19.21
ATOM    719  CG2 ILE A 103      31.135  58.037  90.272  1.00 12.63
ATOM    720  CD1 ILE A 103      31.708  55.185  91.522  1.00 17.58
ATOM    721  N   GLU A 104      33.426  59.429  88.218  1.00 18.81
ATOM    722  CA  GLU A 104      33.369  60.900  88.092  1.00 18.36
ATOM    723  C   GLU A 104      33.828  61.520  86.772  1.00 19.60
ATOM    724  O   GLU A 104      33.420  62.606  86.365  1.00 19.13
ATOM    725  CB  GLU A 104      34.092  61.600  89.241  1.00 18.56
ATOM    726  CG  GLU A 104      33.446  61.448  90.617  1.00 19.21
ATOM    727  CD  GLU A 104      31.994  61.944  90.665  1.00 23.36
ATOM    728  OE1 GLU A 104      31.225  61.359  91.382  1.00 26.94
ATOM    729  OE2 GLU A 104      31.574  62.888  90.013  1.00 28.46
ATOM    730  N   ASP A 105      34.606  60.713  86.049  1.00 18.95
ATOM    731  CA  ASP A 105      34.743  60.936  84.587  1.00 17.40
ATOM    732  C   ASP A 105      33.378  61.099  83.886  1.00 15.12
ATOM    733  O   ASP A 105      33.104  62.102  83.234  1.00 18.61
ATOM    734  CB  ASP A 105      35.429  59.743  83.951  1.00 16.81
ATOM    735  CG  ASP A 105      36.831  59.545  84.440  1.00 15.10
ATOM    736  OD1 ASP A 105      37.573  60.520  84.573  1.00 19.01
ATOM    737  OD2 ASP A 105      37.177  58.402  84.685  1.00 15.56
ATOM    738  N   ALA A 106      32.500  60.091  84.096  1.00 15.05
ATOM    739  CA  ALA A 106      31.111  60.157  83.607  1.00 15.61
ATOM    740  C   ALA A 106      30.166  61.126  84.315  1.00 19.62
ATOM    741  O   ALA A 106      29.409  61.881  83.720  1.00 18.83
ATOM    742  CB  ALA A 106      30.467  58.782  83.682  1.00 11.73
ATOM    743  N   GLY A 107      30.263  61.102  85.674  1.00 21.49
ATOM    744  CA  GLY A 107      29.323  61.899  86.503  1.00 16.83
ATOM    745  C   GLY A 107      29.599  63.356  86.594  1.00 14.80
ATOM    746  O   GLY A 107      28.714  64.204  86.575  1.00 17.67
ATOM    747  N   ASP A 108      30.899  63.611  86.662  1.00 16.37
ATOM    748  CA  ASP A 108      31.305  65.002  86.772  1.00 17.18
ATOM    749  C   ASP A 108      31.877  65.572  85.485  1.00 18.56
ATOM    750  O   ASP A 108      31.324  66.472  84.877  1.00 18.77
ATOM    751  CB  ASP A 108      32.282  65.144  87.947  1.00 16.92
ATOM    752  CG  ASP A 108      32.862  66.530  88.143  1.00 21.34
ATOM    753  OD1 ASP A 108      32.247  67.528  87.812  1.00 23.69
ATOM    754  OD2 ASP A 108      33.983  66.642  88.614  1.00 27.32
ATOM    755  N   LEU A 109      33.049  65.065  85.107  1.00 18.48
ATOM    756  CA  LEU A 109      33.814  65.703  84.005  1.00 20.77
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[Drawing 24]

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ATOM    757  C   LEU A 109      32.954  65.922  82.752  1.00 21.46
ATOM    758  O   LEU A 109      32.905  67.026  82.199  1.00 23.24
ATOM    759  CB  LEU A 109      35.024  64.826  83.679  1.00 20.60
ATOM    760  CG  LEU A 109      36.393  65.285  84.113  1.00 21.65
ATOM    761  CD1 LEU A 109      37.408  64.213  84.096  1.00 17.20
ATOM    762  CD2 LEU A 109      36.469  66.186  85.254  1.00 20.25
ATOM    763  N   ALA A 110      32.206  64.812  82.422  1.00 21.81
ATOM    764  CA  ALA A 110      31.336  64.771  81.215  1.00 20.79
ATOM    765  C   ALA A 110      30.074  65.608  81.232  1.00 23.23
ATOM    766  O   ALA A 110      29.504  65.885  80.182  1.00 23.66
ATOM    767  CB  ALA A 110      30.921  63.333  80.868  1.00 20.49
ATOM    768  N   THR A 111      29.672  66.038  82.465  1.00 20.15
ATOM    769  CA  THR A 111      28.453  66.863  82.653  1.00 19.21
ATOM    770  C   THR A 111      28.654  68.292  83.132  1.00 18.58
ATOM    771  O   THR A 111      27.754  69.106  83.131  1.00 18.77
ATOM    772  CB  THR A 111      27.469  66.257  83.628  1.00 18.46
ATOM    773  OG1 THR A 111      28.011  66.399  84.949  1.00 23.25
ATOM    774  CG2 THR A 111      27.094  64.802  83.347  1.00 15.78
ATOM    775  N   ARG A 112      29.870  68.595  83.547  1.00 20.94
ATOM    776  CA  ARG A 112      30.068  69.805  84.369  1.00 22.62
ATOM    777  C   ARG A 112      29.745  71.185  83.786  1.00 23.68
ATOM    778  O   ARG A 112      29.035  72.025  84.325  1.00 21.09
ATOM    779  CB  ARG A 112      31.512  69.782  84.911  1.00 22.88
ATOM    780  CG  ARG A 112      31.847  70.852  85.952  1.00 22.67
ATOM    781  CD  ARG A 112      33.319  70.922  86.319  1.00 18.55
ATOM    782  NE  ARG A 112      33.831  69.709  86.930  1.00 22.11
ATOM    783  CZ  ARG A 112      35.138  69.496  86.853  1.00 21.99
ATOM    784  NH1 ARG A 112      35.949  70.322  86.227  1.00 23.29
ATOM    785  NH2 ARG A 112      35.623  68.436  87.414  1.00 23.83
ATOM    786  N   SER A 113      30.323  71.398  82.583  1.00 21.66
ATOM    787  CA  SER A 113      30.146  72.736  81.981  1.00 19.14
ATOM    788  C   SER A 113      28.721  73.124  81.629  1.00 20.09
ATOM    789  O   SER A 113      28.288  74.263  81.806  1.00 23.06
ATOM    790  CB  SER A 113      31.029  72.919  80.732  1.00 24.64
ATOM    791  OG  SER A 113      30.812  71.854  79.778  1.00 25.18
ATOM    792  N   ALA A 114      27.955  72.094  81.186  1.00 19.43
ATOM    793  CA  ALA A 114      26.510  72.272  80.944  1.00 17.15
ATOM    794  C   ALA A 114      25.695  72.377  82.247  1.00 16.95
ATOM    795  O   ALA A 114      24.890  73.293  82.402  1.00 17.79
ATOM    796  CB  ALA A 114      25.935  71.096  80.117  1.00 15.05
ATOM    797  N   LYS A 115      25.993  71.462  83.200  1.00 18.76
ATOM    798  CA  LYS A 115      25.431  71.618  84.559  1.00 20.52
ATOM    799  C   LYS A 115      25.524  73.029  85.143  1.00 18.24
ATOM    800  O   LYS A 115      24.535  73.710  85.429  1.00 19.61
ATOM    801  CB  LYS A 115      26.048  70.606  85.508  1.00 17.41
ATOM    802  CG  LYS A 115      25.304  69.294  85.482  1.00 22.93
ATOM    803  CD  LYS A 115      25.867  68.477  86.654  1.00 26.73
ATOM    804  CE  LYS A 115      25.353  67.039  86.850  1.00 26.14
ATOM    805  NZ  LYS A 115      23.888  67.023  87.009  1.00 26.36
ATOM    806  N   ASP A 116      26.784  73.454  85.203  1.00 19.25
ATOM    807  CA  ASP A 116      27.073  74.739  85.832  1.00 20.02
ATOM    808  C   ASP A 116      26.589  75.938  85.076  1.00 23.51
ATOM    809  O   ASP A 116      26.208  76.967  85.612  1.00 24.69
ATOM    810  CB  ASP A 116      28.573  74.896  86.053  1.00 22.26
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[Drawing 25]

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ATOM    811  CG  ASP A 116      29.203  73.871  87.029  1.00 25.80
ATOM    812  OD1 ASP A 116      28.502  73.085  87.657  1.00 27.77
ATOM    813  OD2 ASP A 116      30.431  73.847  87.166  1.00 28.44
ATOM    814  N   HIS A 117      26.596  75.793  83.741  1.00 23.53
ATOM    815  CA  HIS A 117      26.022  76.894  82.957  1.00 21.71
ATOM    816  C   HIS A 117      24.496  77.044  83.026  1.00 20.75
ATOM    817  O   HIS A 117      23.932  78.108  83.223  1.00 20.51
ATOM    818  CB  HIS A 117      26.536  76.787  81.501  1.00 22.93
ATOM    819  CG  HIS A 117      25.987  77.909  80.635  1.00 22.08
ATOM    820  ND1 HIS A 117      26.531  79.128  80.459  1.00 23.33
ATOM    821  CD2 HIS A 117      24.824  77.852  79.881  1.00 22.30
ATOM    822  CE1 HIS A 117      25.741  79.836  79.615  1.00 23.49
ATOM    823  NE2 HIS A 117      24.693  79.041  79.262  1.00 23.57
ATOM    824  N   TYR A 118      23.825  75.906  82.825  1.00 19.95
ATOM    825  CA  TYR A 118      22.363  76.013  82.795  1.00 19.91
ATOM    826  C   TYR A 118      21.711  76.075  84.159  1.00 22.89
ATOM    827  O   TYR A 118      20.615  76.596  84.278  1.00 22.53
ATOM    828  CB  TYR A 118      21.702  74.869  82.020  1.00 18.70
ATOM    829  CG  TYR A 118      22.112  74.964  80.550  1.00 19.86
ATOM    830  CD1 TYR A 118      21.604  76.030  79.775  1.00 20.81
ATOM    831  CD2 TYR A 118      22.998  74.004  80.005  1.00 20.19
ATOM    832  CE1 TYR A 118      21.964  76.104  78.410  1.00 24.18
ATOM    833  CE2 TYR A 118      23.393  74.097  78.652  1.00 22.07
ATOM    834  CZ  TYR A 118      22.841  75.133  77.869  1.00 24.85
ATOM    835  OH  TYR A 118      23.138  75.231  76.525  1.00 25.02
ATOM    836  N   MET A 119      22.385  75.453  85.158  1.00 22.72
ATOM    837  CA  MET A 119      21.795  75.408  86.516  1.00 25.49
ATOM    838  C   MET A 119      20.328  75.033  86.619  1.00 22.79
ATOM    839  O   MET A 119      19.526  75.639  87.309  1.00 23.65
ATOM    840  CB  MET A 119      22.009  76.758  87.200  1.00 31.90
ATOM    841  CG  MET A 119      23.479  77.200  87.296  1.00 41.79
ATOM    842  SD  MET A 119      23.683  78.779  88.163  1.00 50.25
ATOM    843  CE  MET A 119      22.932  79.838  86.910  1.00 48.37
ATOM    844  N   ARG A 120      19.958  74.021  85.840  1.00 21.05
ATOM    845  CA  ARG A 120      18.529  73.782  85.704  1.00 19.45
ATOM    846  C   ARG A 120      17.877  73.247  86.989  1.00 16.34
ATOM    847  O   ARG A 120      18.483  72.369  87.587  1.00 17.21
ATOM    848  CB  ARG A 120      18.345  72.757  84.558  1.00 16.25
ATOM    849  CG  ARG A 120      16.913  72.517  84.063  1.00 17.39
ATOM    850  CD  ARG A 120      16.775  71.558  82.842  1.00 19.83
ATOM    851  NE  ARG A 120      15.450  71.636  82.189  1.00 20.39
ATOM    852  CZ  ARG A 120      14.929  70.642  81.479  1.00 12.14
ATOM    853  NH1 ARG A 120      15.600  69.574  81.259  1.00 12.74
ATOM    854  NH2 ARG A 120      13.724  70.767  81.007  1.00 15.75
ATOM    855  N   ILE A 121      16.676  73.723  87.290  1.00 16.46
ATOM    856  CA  ILE A 121      15.807  73.186  88.360  1.00 22.91
ATOM    857  C   ILE A 121      15.258  71.795  88.080  1.00 23.50
ATOM    858  O   ILE A 121      14.648  71.571  87.043  1.00 26.14
ATOM    859  CB  ILE A 121      14.594  74.134  88.686  1.00 25.98
ATOM    860  CG1 ILE A 121      14.986  75.592  88.783  1.00 29.16
ATOM    861  CG2 ILE A 121      13.793  73.870  89.982  1.00 23.20
ATOM    862  CD1 ILE A 121      16.075  75.914  89.785  1.00 29.73
ATOM    863  N   ARG A 122      15.463  70.877  89.036  1.00 21.01
ATOM    864  CA  ARG A 122      14.883  69.534  88.976  1.00 19.80
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[Drawing 26]

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ATOM    865  C   ARG A 122      13.372  69.532  89.091  1.00 17.61
ATOM    866  O   ARG A 122      12.805  70.488  89.613  1.00 18.79
ATOM    867  CB  ARG A 122      15.533  68.653  90.033  1.00 17.47
ATOM    868  CG  ARG A 122      17.023  68.706  89.841  1.00 18.78
ATOM    869  CD  ARG A 122      17.721  67.861  90.852  1.00 21.00
ATOM    870  NE  ARG A 122      19.141  68.121  90.748  1.00 28.80
ATOM    871  CZ  ARG A 122      19.914  67.081  90.491  1.00 34.31
ATOM    872  NH1 ARG A 122      19.401  65.871  90.426  1.00 40.28
ATOM    873  NH2 ARG A 122      21.196  67.257  90.289  1.00 32.47
ATOM    874  N   PRO A 123      12.700  68.460  88.577  1.00 17.64
ATOM    875  CA  PRO A 123      11.243  68.461  88.684  1.00 18.56
ATOM    876  C   PRO A 123      10.668  68.630  90.118  1.00 20.86
ATOM    877  O   PRO A 123       9.881  69.536  90.331  1.00 20.65
ATOM    878  CB  PRO A 123      10.808  67.150  88.004  1.00 16.86
ATOM    879  CG  PRO A 123      12.028  66.572  87.296  1.00 15.55
ATOM    880  CD  PRO A 123      13.235  67.291  87.881  1.00 15.48
ATOM    881  N   PHE A 124      11.077  67.771  91.105  1.00 21.82
ATOM    882  CA  PHE A 124      10.489  67.948  92.468  1.00 20.01
ATOM    883  C   PHE A 124      10.541  69.394  93.016  1.00 16.73
ATOM    884  O   PHE A 124       9.581  69.970  93.486  1.00 17.82
ATOM    885  CB  PHE A 124      11.044  66.869  93.422  1.00 17.84
ATOM    886  CG  PHE A 124      12.484  67.199  93.795  1.00 19.87
ATOM    887  CD1 PHE A 124      12.748  68.117  94.850  1.00 20.96
ATOM    888  CD2 PHE A 124      13.554  66.632  93.075  1.00 19.87
ATOM    889  CE1 PHE A 124      14.068  68.524  95.134  1.00 21.78
ATOM    890  CE2 PHE A 124      14.881  67.014  93.381  1.00 21.98
ATOM    891  CZ  PHE A 124      15.129  67.975  94.386  1.00 23.27
ATOM    892  N   ALA A 125      11.681  70.039  92.775  1.00 18.30
ATOM    893  CA  ALA A 125      11.866  71.464  93.089  1.00 20.06
ATOM    894  C   ALA A 125      11.033  72.481  92.291  1.00 24.90
ATOM    895  O   ALA A 125      10.455  73.445  92.789  1.00 24.77
ATOM    896  CB  ALA A 125      13.358  71.840  92.990  1.00 16.96
ATOM    897  N   PHE A 126      10.941  72.202  90.977  1.00 23.91
ATOM    898  CA  PHE A 126      10.017  72.958  90.145  1.00 22.66
ATOM    899  C   PHE A 126       8.590  72.919  90.692  1.00 20.74
ATOM    900  O   PHE A 126       7.910  73.945  90.785  1.00 21.66
ATOM    901  CB  PHE A 126      10.051  72.379  88.705  1.00 19.61
ATOM    902  CG  PHE A 126       9.147  73.140  87.765  1.00 16.99
ATOM    903  CD1 PHE A 126       9.669  74.211  87.022  1.00 15.15
ATOM    904  CD2 PHE A 126       7.794  72.757  87.656  1.00 17.75
ATOM    905  CE1 PHE A 126       8.824  74.913  86.144  1.00 14.16
ATOM    906  CE2 PHE A 126       6.940  73.472  86.799  1.00 17.56
ATOM    907  CZ  PHE A 126       7.471  74.538  86.048  1.00 12.93
ATOM    908  N   TYR A 127       8.183  71.664  91.002  1.00 20.13
ATOM    909  CA  TYR A 127       6.843  71.414  91.525  1.00 20.41
ATOM    910  C   TYR A 127       6.642  71.689  93.032  1.00 24.41
ATOM    911  O   TYR A 127       5.525  71.665  93.532  1.00 23.70
ATOM    912  CB  TYR A 127       6.370  69.994  91.207  1.00 20.85
ATOM    913  CG  TYR A 127       6.198  69.850  89.697  1.00 25.51
ATOM    914  CD1 TYR A 127       7.200  69.185  88.947  1.00 27.02
ATOM    915  CD2 TYR A 127       5.064  70.407  89.065  1.00 25.24
ATOM    916  CE1 TYR A 127       7.057  69.037  87.552  1.00 28.75
ATOM    917  CE2 TYR A 127       4.907  70.261  87.663  1.00 28.24
ATOM    918  CZ  TYR A 127       5.891  69.543  86.936  1.00 28.76
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[Drawing 27]

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ATOM    919  OH  TYR A 127       5.716  69.314  85.588  1.00 27.53
ATOM    920  N   GLY A 128       7.764  71.960  93.723  1.00 23.74
ATOM    921  CA  GLY A 128       7.675  72.217  95.170  1.00 24.88
ATOM    922  C   GLY A 128       7.138  71.011  95.924  1.00 24.57
ATOM    923  O   GLY A 128       6.383  71.095  96.866  1.00 29.82
ATOM    924  N   VAL A 129       7.527  69.854  95.426  1.00 24.16
ATOM    925  CA  VAL A 129       7.144  68.604  96.044  1.00 23.21
ATOM    926  C   VAL A 129       8.377  67.802  96.358  1.00 24.99
ATOM    927  O   VAL A 129       9.529  68.188  96.189  1.00 25.90
ATOM    928  CB  VAL A 129       6.193  67.776  95.187  1.00 22.38
ATOM    929  CG1 VAL A 129       6.738  67.390  93.815  1.00 17.75
ATOM    930  CG2 VAL A 129       4.895  68.549  95.125  1.00 24.52
ATOM    931  N   SER A 130       8.089  66.617  96.832  1.00 25.98
ATOM    932  CA  SER A 130       9.242  65.724  96.973  1.00 29.71
ATOM    933  C   SER A 130       9.322  64.672  95.895  1.00 29.73
ATOM    934  O   SER A 130       8.403  64.485  95.103  1.00 30.28
ATOM    935  CB  SER A 130       9.183  64.999  98.309  1.00 35.36
ATOM    936  OG  SER A 130       7.964  64.244  98.427  1.00 41.61
ATOM    937  N   THR A 131      10.440  63.952  95.934  1.00 30.35
ATOM    938  CA  THR A 131      10.533  62.819  94.996  1.00 27.90
ATOM    939  C   THR A 131       9.733  61.569  95.361  1.00 28.80
ATOM    940  O   THR A 131       9.051  61.508  96.366  1.00 30.16
ATOM    941  CB  THR A 131      11.996  62.471  94.783  1.00 26.62
ATOM    942  OG1 THR A 131      12.500  61.841  95.953  1.00 29.21
ATOM    943  CG2 THR A 131      12.839  63.682  94.446  1.00 19.32
ATOM    944  N   CYS A 132       9.835  60.528  94.551  1.00 27.12
ATOM    945  CA  CYS A 132       9.203  59.271  94.996  1.00 27.75
ATOM    946  C   CYS A 132       9.911  58.540  96.137  1.00 28.96
ATOM    947  O   CYS A 132       9.556  57.487  96.634  1.00 28.80
ATOM    948  CB  CYS A 132       9.081  58.274  93.831  1.00 24.68
ATOM    949  SG  CYS A 132      10.538  57.273  93.459  1.00 24.12
ATOM    950  N   ASN A 133      11.021  59.158  96.447  1.00 33.31
ATOM    951  CA  ASN A 133      12.012  58.492  97.236  1.00 39.44
ATOM    952  C   ASN A 133      12.008  58.776  98.750  1.00 45.19
ATOM    953  O   ASN A 133      12.269  57.928  99.583  1.00 50.38
ATOM    954  CB  ASN A 133      13.285  58.876  96.522  1.00 35.83
ATOM    955  CG  ASN A 133      14.294  57.823  96.731  1.00 37.18
ATOM    956  OD1 ASN A 133      15.478  58.059  96.663  1.00 38.83
ATOM    957  ND2 ASN A 133      13.815  56.623  97.017  1.00 42.51
ATOM    958  N   THR A 134      11.642  59.993  99.118  1.00 48.69
ATOM    959  CA  THR A 134      12.585  61.110  98.925  1.00 56.17
ATOM    960  C   THR A 134      13.935  61.171  99.735  1.00 61.49
ATOM    961  OCT1 THR A 134      14.052  60.565 100.816  1.00 66.36
ATOM    962  OCT2 THR A 134      14.937  61.779  99.291  1.00 63.18
ATOM    963  CB  THR A 134      11.704  62.374  98.968  1.00 56.04
ATOM    964  OG1 THR A 134      12.306  63.614  98.469  1.00 56.23
ATOM    965  CG2 THR A 134      10.869  62.400 100.243  1.00 53.77
ATOM    966  N   GLN A 137      16.953  60.437 100.819  1.00100.00
ATOM    967  CA  GLN A 137      17.845  60.498 102.027  1.00 99.78
ATOM    968  C   GLN A 137      19.036  61.512 102.211  1.00 98.61
ATOM    969  O   GLN A 137      19.386  61.891 103.324  1.00 97.54
ATOM    970  CB  GLN A 137      18.343  59.083 102.397  1.00100.00
ATOM    971  CG  GLN A 137      17.669  58.407 103.615  1.00 99.38
ATOM    972  CD  GLN A 137      18.060  59.017 104.963  1.00 98.64
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[Drawing 28]

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ATOM    973  OE1 GLN A 137      17.484  58.784 106.009  1.00 98.03
ATOM    974  NE2 GLN A 137      19.086  59.847 104.972  1.00100.00
ATOM    975  N   ASP A 138      19.627  61.949 101.081  1.00 97.91
ATOM    976  CA  ASP A 138      20.479  63.151 101.162  1.00 95.99
ATOM    977  C   ASP A 138      19.761  64.494 101.063  1.00 93.86
ATOM    978  O   ASP A 138      18.589  64.579 100.711  1.00 92.76
ATOM    979  CB  ASP A 138      21.585  63.102 100.115  1.00 97.46
ATOM    980  CG  ASP A 138      22.893  62.963 100.866  1.00100.00
ATOM    981  OD1 ASP A 138      23.371  61.842 101.028  1.00100.00
ATOM    982  OD2 ASP A 138      23.432  63.969 101.333  1.00100.00
ATOM    983  N   LYS A 139      20.494  65.564 101.380  1.00 92.69
ATOM    984  CA  LYS A 139      19.813  66.855 101.218  1.00 91.50
ATOM    985  C   LYS A 139      19.719  67.313  99.775  1.00 88.92
ATOM    986  O   LYS A 139      20.557  68.082  99.308  1.00 89.47
ATOM    987  CB  LYS A 139      20.464  67.987 102.010  1.00 94.21
ATOM    988  CG  LYS A 139      19.574  69.246 102.020  1.00 96.82
ATOM    989  CD  LYS A 139      20.362  70.569 102.123  1.00 99.43
ATOM    990  CE  LYS A 139      20.785  71.241 100.793  1.00100.00
ATOM    991  NZ  LYS A 139      21.686  70.402  99.978  1.00100.00
ATOM    992  N   LEU A 140      18.654  66.828  99.114  1.00 85.87
ATOM    993  CA  LEU A 140      18.426  67.018  97.660  1.00 79.60
ATOM    994  C   LEU A 140      18.772  68.392  97.043  1.00 75.62
ATOM    995  O   LEU A 140      18.242  69.442  97.416  1.00 75.74
ATOM    996  CB  LEU A 140      16.981  66.620  97.283  1.00 77.27
ATOM    997  CG  LEU A 140      16.640  65.142  97.462  1.00 74.31
ATOM    998  CD1 LEU A 140      17.569  64.263  96.645  1.00 73.21
ATOM    999  CD2 LEU A 140      15.178  64.838  97.167  1.00 73.91
ATOM   1000  N   SER A 141      19.713  68.357  96.067  1.00 70.08
ATOM   1001  CA  SER A 141      19.868  69.619  95.320  1.00 64.86
ATOM   1002  C   SER A 141      18.713  69.941  94.396  1.00 61.18
ATOM   1003  O   SER A 141      18.189  69.080  93.708  1.00 61.77
ATOM   1004  CB  SER A 141      21.127  69.682  94.461  1.00 64.49
ATOM   1005  OG  SER A 141      21.354  71.028  94.002  1.00 66.43
ATOM   1006  N   LYS A 142      18.349  71.235  94.379  1.00 57.81
ATOM   1007  CA  LYS A 142      17.238  71.661  93.493  1.00 54.35
ATOM   1008  C   LYS A 142      17.498  71.829  91.964  1.00 45.94
ATOM   1009  O   LYS A 142      16.615  72.091  91.202  1.00 40.89
ATOM   1010  CB  LYS A 142      16.631  72.953  94.061  1.00 57.69
ATOM   1011  CG  LYS A 142      17.518  74.222  93.959  1.00 62.63
ATOM   1012  CD  LYS A 142      16.625  75.420  93.545  1.00 68.07
ATOM   1013  CE  LYS A 142      17.200  76.856  93.475  1.00 71.28
ATOM   1014  NZ  LYS A 142      16.136  77.844  93.162  1.00 70.88
ATOM   1015  N   ASN A 143      18.772  71.719  91.611  1.00 45.99
ATOM   1016  CA  ASN A 143      19.527  72.392  90.538  1.00 44.71
ATOM   1017  C   ASN A 143      20.592  71.481  89.878  1.00 42.57
ATOM   1018  O   ASN A 143      20.794  70.322  90.283  1.00 40.17
ATOM   1019  CB  ASN A 143      20.343  73.547  91.124  1.00 50.34
ATOM   1020  CG  ASN A 143      19.624  74.818  90.876  1.00 55.19
ATOM   1021  OD1 ASN A 143      18.441  74.944  91.098  1.00 59.21
ATOM   1022  ND2 ASN A 143      20.366  75.786  90.389  1.00 59.88
ATOM   1023  N   GLY A 144      21.265  72.084  88.819  1.00 39.72
ATOM   1024  CA  GLY A 144      22.264  71.401  87.958  1.00 26.74
ATOM   1025  C   GLY A 144      21.691  70.105  87.407  1.00 21.44
ATOM   1026  O   GLY A 144      22.343  69.071  87.322  1.00 25.78
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[Drawing 29]

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ATOM   1027  N   SER A 145      20.380  70.181  87.105  1.00 19.15
ATOM   1028  CA  SER A 145      19.590  69.023  86.596  1.00 20.14
ATOM   1029  C   SER A 145      20.077  68.461  85.223  1.00 20.58
ATOM   1030  O   SER A 145      20.121  67.279  84.943  1.00 21.22
ATOM   1031  CB  SER A 145      18.171  69.499  86.338  1.00 18.89
ATOM   1032  OG  SER A 145      17.219  68.526  86.712  1.00 28.02
ATOM   1033  N   TYR A 146      20.531  69.419  84.399  1.00 22.70
ATOM   1034  CA  TYR A 146      20.867  69.170  82.968  1.00 19.51
ATOM   1035  C   TYR A 146      22.366  69.260  82.633  1.00 16.00
ATOM   1036  O   TYR A 146      22.930  70.339  82.736  1.00 17.85
ATOM   1037  CB  TYR A 146      20.097  70.216  82.114  1.00 18.79
ATOM   1038  CG  TYR A 146      20.211  69.933  80.600  1.00 20.29
ATOM   1039  CD1 TYR A 146      19.310  69.027  80.015  1.00 19.58
ATOM   1040  CD2 TYR A 146      21.205  70.582  79.824  1.00 20.42
ATOM   1041  CE1 TYR A 146      19.389  68.781  78.631  1.00 23.13
ATOM   1042  CE2 TYR A 146      21.287  70.337  78.424  1.00 21.68
ATOM   1043  CZ  TYR A 146      20.349  69.449  77.849  1.00 20.03
ATOM   1044  OH  TYR A 146      20.320  69.236  76.483  1.00 21.40
ATOM   1045  N   PRO A 147      22.994  68.144  82.187  1.00 16.52
ATOM   1046  CA  PRO A 147      22.385  66.793  82.188  1.00 17.87
ATOM   1047  C   PRO A 147      22.496  66.069  83.580  1.00 20.67
ATOM   1048  O   PRO A 147      23.198  66.531  84.480  1.00 22.35
ATOM   1049  CB  PRO A 147      23.250  66.107  81.122  1.00 15.36
ATOM   1050  CG  PRO A 147      24.649  66.716  81.297  1.00 15.06
ATOM   1051  CD  PRO A 147      24.356  68.168  81.630  1.00 16.28
ATOM   1052  N   SER A 148      21.827  64.908  83.662  1.00 18.73
ATOM   1053  CA  SER A 148      21.951  64.019  84.823  1.00 19.51
ATOM   1054  C   SER A 148      23.269  63.281  84.930  1.00 19.93
ATOM   1055  O   SER A 148      23.601  62.439  84.108  1.00 20.09
ATOM   1056  CB  SER A 148      20.828  62.997  84.807  1.00 18.55
ATOM   1057  OG  SER A 148      20.990  61.966  85.780  1.00 19.29
ATOM   1058  N   GLY A 149      24.036  63.618  85.972  1.00 18.26
ATOM   1059  CA  GLY A 149      25.284  62.888  86.231  1.00 16.00
ATOM   1060  C   GLY A 149      25.096  61.411  86.577  1.00 19.38
ATOM   1061  O   GLY A 149      25.791  60.549  86.044  1.00 21.04
ATOM   1062  N   HIS A 150      24.085  61.096  87.427  1.00 20.25
ATOM   1063  CA  HIS A 150      23.690  59.680  87.624  1.00 19.01
ATOM   1064  C   HIS A 150      23.381  58.861  86.330  1.00 19.93
ATOM   1065  O   HIS A 150      23.833  57.725  86.145  1.00 20.78
ATOM   1066  CB  HIS A 150      22.507  59.533  88.619  1.00 18.01
ATOM   1067  CG  HIS A 150      22.162  58.068  88.909  1.00 20.60
ATOM   1068  ND1 HIS A 150      22.864  57.235  89.730  1.00 23.41
ATOM   1069  CD2 HIS A 150      21.117  57.313  88.374  1.00 21.91
ATOM   1070  CE1 HIS A 150      22.287  55.996  89.732  1.00 22.83
ATOM   1071  NE2 HIS A 150      21.220  56.051  88.893  1.00 24.19
ATOM   1072  N   THR A 151      22.593  59.482  85.432  1.00 18.98
ATOM   1073  CA  THR A 151      22.325  58.814  84.132  1.00 17.17
ATOM   1074  C   THR A 151      23.548  58.652  83.228  1.00 13.61
ATOM   1075  O   THR A 151      23.814  57.594  82.659  1.00 17.00
ATOM   1076  CB  THR A 151      21.270  59.590  83.407  1.00 16.57
ATOM   1077  OG1 THR A 151      20.137  59.738  84.258  1.00 18.00
ATOM   1078  CG2 THR A 151      20.898  58.983  82.045  1.00 13.22
ATOM   1079  N   SER A 152      24.361  59.722  83.197  1.00 14.30
ATOM   1080  CA  SER A 152      25.687  59.598  82.557  1.00 15.99
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[Drawing 30]

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ATOM   1081  C   SER A 152      26.575  58.446  83.029  1.00 19.72
ATOM   1082  O   SER A 152      27.086  57.628  82.255  1.00 17.38
ATOM   1083  CB  SER A 152      26.434  60.927  82.644  1.00 12.58
ATOM   1084  OG  SER A 152      27.648  60.861  81.911  1.00 16.08
ATOM   1085  N   ILE A 153      26.662  58.340  84.403  1.00 20.60
ATOM   1086  CA  ILE A 153      27.272  57.121  85.017  1.00 16.15
ATOM   1087  C   ILE A 153      26.622  55.802  84.631  1.00 10.76
ATOM   1088  O   ILE A 153      27.293  54.850  84.262  1.00 14.38
ATOM   1089  CB  ILE A 153      27.384  57.170  86.608  1.00 15.02
ATOM   1090  CG1 ILE A 153      28.187  58.421  86.963  1.00 16.37
ATOM   1091  CG2 ILE A 153      28.154  55.944  87.164  1.00 12.98
ATOM   1092  CD1 ILE A 153      27.870  59.034  88.338  1.00 16.58
ATOM   1093  N   GLY A 154      25.285  55.763  84.720  1.00  9.72
ATOM   1094  CA  GLY A 154      24.662  54.476  84.397  1.00 13.63
ATOM   1095  C   GLY A 154      24.843  54.033  82.910  1.00 16.00
ATOM   1096  O   GLY A 154      25.022  52.866  82.571  1.00 16.37
ATOM   1097  N   TRP A 155      24.801  55.047  82.025  1.00 16.77
ATOM   1098  CA  TRP A 155      24.960  54.747  80.589  1.00 16.73
ATOM   1099  C   TRP A 155      26.378  54.351  80.200  1.00 15.90
ATOM   1100  O   TRP A 155      26.656  53.301  79.628  1.00 18.41
ATOM   1101  CB  TRP A 155      24.442  55.940  79.771  1.00 16.52
ATOM   1102  CG  TRP A 155      24.320  55.475  78.321  1.00 19.11
ATOM   1103  CD1 TRP A 155      25.009  56.015  77.210  1.00 18.94
ATOM   1104  CD2 TRP A 155      23.471  54.426  77.765  1.00 18.98
ATOM   1105  NE1 TRP A 155      24.679  55.352  76.044  1.00 17.77
ATOM   1106  CE2 TRP A 155      23.781  54.322  76.357  1.00 22.22
ATOM   1107  CE3 TRP A 155      22.585  53.486  78.337  1.00 19.43
ATOM   1108  CZ2 TRP A 155      23.084  53.377  75.564  1.00 17.99
ATOM   1109  CZ3 TRP A 155      21.913  52.538  77.537  1.00 19.46
ATOM   1110  CH2 TRP A 155      22.191  52.464  76.158  1.00 17.84
ATOM   1111  N   ALA A 156      27.299  55.209  80.623  1.00 15.37
ATOM   1112  CA  ALA A 156      28.702  54.836  80.515  1.00 14.36
ATOM   1113  C   ALA A 156      29.156  53.503  81.108  1.00 19.46
ATOM   1114  O   ALA A 156      29.895  52.723  80.528  1.00 19.86
ATOM   1115  CB  ALA A 156      29.564  55.918  81.136  1.00 15.83
ATOM   1116  N   THR A 157      28.651  53.207  82.327  1.00 19.95
ATOM   1117  CA  THR A 157      28.820  51.832  82.831  1.00 17.52
ATOM   1118  C   THR A 157      28.177  50.744  81.994  1.00 15.22
ATOM   1119  O   THR A 157      28.825  49.745  81.765  1.00 19.09
ATOM   1120  CB  THR A 157      28.328  51.667  84.291  1.00 14.92
ATOM   1121  OG1 THR A 157      28.932  52.679  85.054  1.00 18.29
ATOM   1122  CG2 THR A 157      28.620  50.327  84.944  1.00 13.21
ATOM   1123  N   ALA A 158      26.930  50.947  81.535  1.00 14.63
ATOM   1124  CA  ALA A 158      26.365  49.936  80.621  1.00 17.10
ATOM   1125  C   ALA A 158      27.213  49.686  79.354  1.00 15.52
ATOM   1126  O   ALA A 158      27.539  48.565  79.025  1.00 16.52
ATOM   1127  CB  ALA A 158      24.942  50.300  80.203  1.00 13.99
ATOM   1128  N   LEU A 159      27.655  50.766  78.705  1.00 17.91
ATOM   1129  CA  LEU A 159      28.613  50.615  77.580  1.00 17.69
ATOM   1130  C   LEU A 159      29.895  49.851  77.846  1.00 19.93
ATOM   1131  O   LEU A 159      30.277  48.954  77.092  1.00 19.21
ATOM   1132  CB  LEU A 159      28.959  51.971  76.939  1.00 14.08
ATOM   1133  CG  LEU A 159      27.744  52.759  76.396  1.00 11.97
ATOM   1134  CD1 LEU A 159      27.045  52.105  75.210  1.00 12.90
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[Drawing 31]

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ATOM   1135  CD2 LEU A 159      28.177  54.158  76.046  1.00 12.40
ATOM   1136  N   VAL A 160      30.547  50.181  79.014  1.00 21.70
ATOM   1137  CA  VAL A 160      31.713  49.315  79.310  1.00 20.85
ATOM   1138  C   VAL A 160      31.429  47.877  79.767  1.00 21.33
ATOM   1139  O   VAL A 160      32.086  46.918  79.409  1.00 20.74
ATOM   1140  CB  VAL A 160      32.574  50.032  80.367  1.00 21.59
ATOM   1141  CG1 VAL A 160      33.518  49.143  81.174  1.00 20.11
ATOM   1142  CG2 VAL A 160      33.299  51.307  79.977  1.00 20.10
ATOM   1143  N   LEU A 161      30.351  47.711  80.542  1.00 21.86
ATOM   1144  CA  LEU A 161      29.850  46.366  80.838  1.00 20.50
ATOM   1145  C   LEU A 161      29.462  45.490  79.613  1.00 23.16
ATOM   1146  O   LEU A 161      29.753  44.294  79.564  1.00 22.89
ATOM   1147  CB  LEU A 161      28.623  46.472  81.733  1.00 18.65
ATOM   1148  CG  LEU A 161      28.685  46.438  83.262  1.00 20.99
ATOM   1149  CD1 LEU A 161      27.473  46.378  84.194  1.00 21.43
ATOM   1150  CD2 LEU A 161      29.729  45.492  83.855  1.00 19.30
ATOM   1151  N   ALA A 162      28.755  46.146  78.648  1.00 21.20
ATOM   1152  CA  ALA A 162      28.384  45.496  77.361  1.00 19.14
ATOM   1153  C   ALA A 162      29.591  44.948  76.586  1.00 17.15
ATOM   1154  O   ALA A 162      29.620  43.812  76.133  1.00 20.85
ATOM   1155  CB  ALA A 162      27.581  46.464  76.500  1.00 17.38
ATOM   1156  N   GLU A 163      30.663  45.745  76.603  1.00 14.74
ATOM   1157  CA  GLU A 163      31.962  45.262  76.118  1.00 17.48
ATOM   1158  C   GLU A 163      32.648  44.080  76.824  1.00 22.07
ATOM   1159  O   GLU A 163      33.271  43.216  76.227  1.00 23.96
ATOM   1160  CB  GLU A 163      32.915  46.448  76.000  1.00 13.52
ATOM   1161  CG  GLU A 163      34.227  46.018  75.359  1.00 13.04
ATOM   1162  CD  GLU A 163      35.240  47.119  75.338  1.00 15.88
ATOM   1163  OE1 GLU A 163      36.427  46.814  75.269  1.00 19.78
ATOM   1164  OE2 GLU A 163      34.873  48.290  75.377  1.00 20.10
ATOM   1165  N   ILE A 164      32.504  44.039  78.153  1.00 20.17
ATOM   1166  CA  ILE A 164      32.996  42.869  78.905  1.00 18.72
ATOM   1167  C   ILE A 164      32.164  41.609  78.757  1.00 17.39
ATOM   1168  O   ILE A 164      32.635  40.481  78.674  1.00 20.44
ATOM   1169  CB  ILE A 164      33.132  43.293  80.382  1.00 20.67
ATOM   1170  CG1 ILE A 164      34.222  44.361  80.452  1.00 18.83
ATOM   1171  CG2 ILE A 164      33.398  42.110  81.345  1.00 20.70
ATOM   1172  CD1 ILE A 164      34.144  45.084  81.793  1.00 21.31
ATOM   1173  N   ASN A 165      30.869  41.846  78.704  1.00 18.41
ATOM   1174  CA  ASN A 165      29.979  40.712  78.524  1.00 21.36
ATOM   1175  C   ASN A 165      28.957  40.867  77.375  1.00 23.74
ATOM   1176  O   ASN A 165      27.753  40.988  77.563  1.00 23.14
ATOM   1177  CB  ASN A 165      29.324  40.404  79.878  1.00 21.69
ATOM   1178  CG  ASN A 165      28.471  39.156  79.861  1.00 25.72
ATOM   1179  OD1 ASN A 165      28.469  38.307  78.967  1.00 29.37
ATOM   1180  ND2 ASN A 165      27.730  39.051  80.951  1.00 27.39
ATOM   1181  N   PRO A 166      29.466  40.814  76.118  1.00 25.93
ATOM   1182  CA  PRO A 166      28.556  40.971  74.957  1.00 26.27
ATOM   1183  C   PRO A 166      27.447  39.924  74.837  1.00 24.06
ATOM   1184  O   PRO A 166      26.361  40.184  74.360  1.00 24.11
ATOM   1185  CB  PRO A 166      29.517  40.940  73.781  1.00 25.15
ATOM   1186  CG  PRO A 166      30.731  40.254  74.255  1.00 27.68
ATOM   1187  CD  PRO A 166      30.849  40.613  75.728  1.00 24.89
ATOM   1188  N   GLN A 167      27.679  38.741  75.385  1.00 25.15
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[Drawing 32]

ATOM	1189	CA	GLN	A	167	26.552	37.804	75.508	1.00 27.09
ATOM	1190	C	GLN	A	167	25.275	38.321	76.189	1.00 25.06
ATOM	1191	O	GLN	A	167	24.162	37.932	75.883	1.00 23.75
ATOM	1192	CB	GLN	A	167	27.080	36.617	76.282	1.00 35.50
ATOM	1193	CG	GLN	A	167	27.386	35.380	75.468	1.00 52.12
ATOM	1194	CD	GLN	A	167	26.074	34.747	75.000	1.00 65.78
ATOM	1195	OE1	GLN	A	167	25.950	34.245	73.889	1.00 72.02
ATOM	1196	NE2	GLN	A	167	25.047	34.780	75.867	1.00 71.50
ATOM	1197	N	ARG	A	168	25.513	39.242	77.156	1.00 26.04
ATOM	1198	CA	ARG	A	168	24.389	39.900	77.837	1.00 25.63
ATOM	1199	C	ARG	A	168	24.242	41.368	77.558	1.00 23.88
ATOM	1200	O	ARG	A	168	23.632	42.106	78.308	1.00 24.09
ATOM	1201	CB	ARG	A	168	24.452	39.638	79.337	1.00 24.63
ATOM	1202	CG	ARG	A	168	24.087	38.171	79.408	1.00 25.72
ATOM	1203	CD	ARG	A	168	23.986	37.617	80.802	1.00 33.72
ATOM	1204	NE	ARG	A	168	22.970	38.302	81.587	1.00 35.38
ATOM	1205	CZ	ARG	A	168	21.729	37.877	81.680	1.00 35.91
ATOM	1206	NH1	ARG	A	168	20.908	38.482	82.490	1.00 34.99
ATOM	1207	NH2	ARG	A	168	21.314	36.867	80.975	1.00 39.28
ATOM	1208	N	GLN	A	169	24.838	41.810	76.424	1.00 23.22
ATOM	1209	CA	GLN	A	169	24.820	43.228	76.080	1.00 19.99
ATOM	1210	C	GLN	A	169	23.441	43.850	76.067	1.00 21.38
ATOM	1211	O	GLN	A	169	23.216	44.969	76.492	1.00 24.60
ATOM	1212	CB	GLN	A	169	25.571	43.510	74.769	1.00 22.07
ATOM	1213	CG	GLN	A	169	24.970	42.878	73.494	1.00 20.93
ATOM	1214	CD	GLN	A	169	25.716	43.287	72.207	1.00 23.42
ATOM	1215	OE1	GLN	A	169	26.680	44.055	72.202	1.00 24.82
ATOM	1216	NE2	GLN	A	169	25.186	42.738	71.109	1.00 13.86
ATOM	1217	N	ASN	A	170	22.455	43.089	75.600	1.00 20.48
ATOM	1218	CA	ASN	A	170	21.138	43.736	75.525	1.00 20.97
ATOM	1219	C	ASN	A	170	20.484	44.007	76.892	1.00 19.73
ATOM	1220	O	ASN	A	170	19.852	45.020	77.128	1.00 18.90
ATOM	1221	CB	ASN	A	170	20.195	42.930	74.595	1.00 24.43
ATOM	1222	CG	ASN	A	170	20.763	42.881	73.153	1.00 25.82
ATOM	1223	OD1	ASN	A	170	20.842	43.862	72.440	1.00 26.22
ATOM	1224	ND2	ASN	A	170	21.197	41.709	72.734	1.00 25.47
ATOM	1225	N	GLU	A	171	20.680	43.042	77.790	1.00 21.27
ATOM	1226	CA	GLU	A	171	20.166	43.146	79.157	1.00 19.84
ATOM	1227	C	GLU	A	171	20.849	44.220	79.926	1.00 16.23
ATOM	1228	O	GLU	A	171	20.199	45.026	80.575	1.00 20.87
ATOM	1229	CB	GLU	A	171	20.317	41.822	79.892	1.00 20.47
ATOM	1230	CG	GLU	A	171	19.412	40.750	79.312	1.00 24.15
ATOM	1231	CD	GLU	A	171	20.157	39.825	78.376	1.00 26.37
ATOM	1232	OE1	GLU	A	171	21.076	40.256	77.685	1.00 23.54
ATOM	1233	OE2	GLU	A	171	19.801	38.645	78.363	1.00 32.14
ATOM	1234	N	ILE	A	172	22.169	44.236	79.751	1.00 17.53
ATOM	1235	CA	ILE	A	172	23.029	45.318	80.257	1.00 16.90
ATOM	1236	C	ILE	A	172	22.679	46.733	79.813	1.00 20.21
ATOM	1237	O	ILE	A	172	22.454	47.636	80.617	1.00 18.91
ATOM	1238	CB	ILE	A	172	24.507	44.992	79.956	1.00 17.00
ATOM	1239	CG1	ILE	A	172	25.000	43.685	80.613	1.00 15.78
ATOM	1240	CG2	ILE	A	172	25.426	46.163	80.300	1.00 18.13
ATOM	1241	CD1	ILE	A	172	26.426	43.320	80.163	1.00 13.83
ATOM	1242	N	LEU	A	173	22.575	46.909	78.468	1.00 20.75

[Drawing 33]

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ATOM   1243  CA  LEU A 173      22.107  48.206  77.946  1.00 17.70
ATOM   1244  C   LEU A 173      20.699  48.622  78.396  1.00 16.26
ATOM   1245  O   LEU A 173      20.376  49.774  78.663  1.00 18.18
ATOM   1246  CB  LEU A 173      22.176  48.201  76.419  1.00 16.81
ATOM   1247  CG  LEU A 173      23.600  48.021  75.966  1.00 18.73
ATOM   1248  CD1 LEU A 173      24.410  49.282  76.138  1.00 16.97
ATOM   1249  CD2 LEU A 173      23.619  47.550  74.505  1.00 23.91
ATOM   1250  N   LYS A 174      19.835  47.625  78.475  1.00 15.86
ATOM   1251  CA  LYS A 174      18.494  47.970  78.945  1.00 19.21
ATOM   1252  C   LYS A 174      18.453  48.415  80.429  1.00 21.86
ATOM   1253  O   LYS A 174      17.800  49.404  80.737  1.00 20.00
ATOM   1254  CB  LYS A 174      17.577  46.773  78.700  1.00 20.19
ATOM   1255  CG  LYS A 174      16.094  47.130  78.731  1.00 27.84
ATOM   1256  CD  LYS A 174      15.799  48.368  77.858  1.00 36.14
ATOM   1257  CE  LYS A 174      14.309  48.628  77.774  1.00 40.56
ATOM   1258  NZ  LYS A 174      13.775  48.622  79.156  1.00 49.45
ATOM   1259  N   ARG A 175      19.250  47.698  81.286  1.00 21.93
ATOM   1260  CA  ARG A 175      19.476  48.145  82.686  1.00 19.65
ATOM   1261  C   ARG A 175      20.037  49.561  82.807  1.00 16.70
ATOM   1262  O   ARG A 175      19.476  50.419  83.468  1.00 18.12
ATOM   1263  CB  ARG A 175      20.345  47.126  83.467  1.00 21.25
ATOM   1264  CG  ARG A 175      20.608  47.477  84.954  1.00 22.00
ATOM   1265  CD  ARG A 175      19.259  47.669  85.656  1.00 25.63
ATOM   1266  NE  ARG A 175      19.435  47.923  87.095  1.00 29.77
ATOM   1267  CZ  ARG A 175      18.601  48.672  87.834  1.00 27.58
ATOM   1268  NH1 ARG A 175      17.550  49.301  87.355  1.00 24.74
ATOM   1269  NH2 ARG A 175      18.792  48.780  89.073  1.00 27.45
ATOM   1270  N   GLY A 176      21.122  49.800  82.048  1.00 17.53
ATOM   1271  CA  GLY A 176      21.698  51.143  81.893  1.00 17.18
ATOM   1272  C   GLY A 176      20.679  52.214  81.552  1.00 21.18
ATOM   1273  O   GLY A 176      20.582  53.278  82.149  1.00 21.85
ATOM   1274  N   TYR A 177      19.847  51.887  80.547  1.00 22.48
ATOM   1275  CA  TYR A 177      18.756  52.787  80.117  1.00 20.51
ATOM   1276  C   TYR A 177      17.754  53.106  81.242  1.00 18.92
ATOM   1277  O   TYR A 177      17.406  54.246  81.542  1.00 15.84
ATOM   1278  CB  TYR A 177      18.007  52.120  78.939  1.00 23.76
ATOM   1279  CG  TYR A 177      17.210  53.131  78.177  1.00 24.47
ATOM   1280  CD1 TYR A 177      15.817  53.306  78.397  1.00 26.61
ATOM   1281  CD2 TYR A 177      17.941  53.869  77.236  1.00 29.40
ATOM   1282  CE1 TYR A 177      15.139  54.308  77.661  1.00 29.08
ATOM   1283  CE2 TYR A 177      17.270  54.855  76.515  1.00 30.70
ATOM   1284  CZ  TYR A 177      15.899  55.092  76.747  1.00 32.01
ATOM   1285  OH  TYR A 177      15.401  56.167  76.020  1.00 41.52
ATOM   1286  N   GLU A 178      17.354  52.008  81.867  1.00 19.45
ATOM   1287  CA  GLU A 178      16.429  52.094  82.972  1.00 22.02
ATOM   1288  C   GLU A 178      16.820  52.802  84.236  1.00 20.09
ATOM   1289  O   GLU A 178      16.001  53.492  84.805  1.00 21.41
ATOM   1290  CB  GLU A 178      16.010  50.731  83.357  1.00 25.45
ATOM   1291  CG  GLU A 178      15.173  50.032  82.303  1.00 34.73
ATOM   1292  CD  GLU A 178      13.893  50.810  81.951  1.00 40.36
ATOM   1293  OE1 GLU A 178      13.432  51.667  82.707  1.00 33.51
ATOM   1294  OE2 GLU A 178      13.352  50.556  80.876  1.00 45.77
ATOM   1295  N   LEU A 179      18.090  52.695  84.609  1.00 19.63
ATOM   1296  CA  LEU A 179      18.655  53.567  85.665  1.00 19.10
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[Drawing 34]

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ATOM   1297  C   LEU A 179      18.366  55.051  85.511  1.00 20.25
ATOM   1298  O   LEU A 179      17.838  55.726  86.374  1.00 19.26
ATOM   1299  CB  LEU A 179      20.178  53.361  85.813  1.00 17.45
ATOM   1300  CG  LEU A 179      20.610  52.013  86.426  1.00 18.23
ATOM   1301  CD1 LEU A 179      20.093  51.829  87.873  1.00 16.98
ATOM   1302  CD2 LEU A 179      22.124  51.811  86.340  1.00 16.34
ATOM   1303  N   GLY A 180      18.647  55.545  84.290  1.00 19.84
ATOM   1304  CA  GLY A 180      18.171  56.903  83.987  1.00 15.90
ATOM   1305  C   GLY A 180      16.655  57.072  84.027  1.00 17.05
ATOM   1306  O   GLY A 180      16.130  58.031  84.564  1.00 19.49
ATOM   1307  N   GLN A 181      15.923  56.068  83.478  1.00 18.19
ATOM   1308  CA  GLN A 181      14.448  56.207  83.522  1.00 17.92
ATOM   1309  C   GLN A 181      13.822  56.265  84.938  1.00 18.20
ATOM   1310  O   GLN A 181      12.903  57.008  85.258  1.00 19.66
ATOM   1311  CB  GLN A 181      13.716  55.189  82.620  1.00 15.39
ATOM   1312  CG  GLN A 181      13.936  55.324  81.073  1.00 15.18
ATOM   1313  CD  GLN A 181      13.823  56.763  80.634  1.00 13.30
ATOM   1314  OE1 GLN A 181      14.769  57.400  80.176  1.00 17.23
ATOM   1315  NE2 GLN A 181      12.626  57.300  80.812  1.00 12.82
ATOM   1316  N   SER A 182      14.440  55.497  85.821  1.00 20.03
ATOM   1317  CA  SER A 182      14.156  55.600  87.273  1.00 16.56
ATOM   1318  C   SER A 182      14.209  56.973  87.871  1.00 14.78
ATOM   1319  O   SER A 182      13.305  57.369  88.582  1.00 19.49
ATOM   1320  CB  SER A 182      15.056  54.630  88.021  1.00 15.31
ATOM   1321  OG  SER A 182      14.563  53.332  87.714  1.00 16.70
ATOM   1322  N   ARG A 183      15.246  57.738  87.523  1.00 16.99
ATOM   1323  CA  ARG A 183      15.289  59.169  87.858  1.00 15.54
ATOM   1324  C   ARG A 183      14.161  60.085  87.309  1.00 19.55
ATOM   1325  O   ARG A 183      13.693  61.039  87.956  1.00 17.04
ATOM   1326  CB  ARG A 183      16.661  59.785  87.569  1.00 14.08
ATOM   1327  CG  ARG A 183      17.811  59.024  88.265  1.00 19.49
ATOM   1328  CD  ARG A 183      17.716  59.007  89.816  1.00 21.46
ATOM   1329  NE  ARG A 183      18.519  57.930  90.434  1.00 22.16
ATOM   1330  CZ  ARG A 183      19.509  58.121  91.300  1.00 19.15
ATOM   1331  NH1 ARG A 183      19.913  59.335  91.541  1.00 16.50
ATOM   1332  NH2 ARG A 183      20.042  57.081  91.914  1.00 18.34
ATOM   1333  N   VAL A 184      13.681  59.704  86.076  1.00 21.38
ATOM   1334  CA  VAL A 184      12.494  60.417  85.589  1.00 19.02
ATOM   1335  C   VAL A 184      11.194  60.051  86.301  1.00 16.98
ATOM   1336  O   VAL A 184      10.468  60.913  86.800  1.00 18.45
ATOM   1337  CB  VAL A 184      12.368  60.194  84.070  1.00 19.28
ATOM   1338  CG1 VAL A 184      11.057  60.717  83.486  1.00 17.84
ATOM   1339  CG2 VAL A 184      13.517  60.605  83.169  1.00 15.69
ATOM   1340  N   ILE A 185      10.977  58.734  86.408  1.00 17.32
ATOM   1341  CA  ILE A 185       9.834  58.241  87.202  1.00 21.14
ATOM   1342  C   ILE A 185       9.790  58.797  88.672  1.00 22.77
ATOM   1343  O   ILE A 185       8.749  59.230  89.142  1.00 22.95
ATOM   1344  CB  ILE A 185       9.810  56.695  87.193  1.00 20.65
ATOM   1345  CG1 ILE A 185       9.555  56.195  85.758  1.00 18.43
ATOM   1346  CG2 ILE A 185       8.776  56.133  88.202  1.00 18.29
ATOM   1347  CD1 ILE A 185       9.914  54.734  85.494  1.00 14.60
ATOM   1348  N   CYS A 186      10.976  58.837  89.332  1.00 20.34
ATOM   1349  CA  CYS A 186      11.005  59.262  90.745  1.00 21.26
ATOM   1350  C   CYS A 186      10.979  60.766  90.931  1.00 22.80
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[Drawing 35]

ATOM	1351	O	CYS	A	186	10.775	61.304	92.009	1.00 23.98
ATOM	1352	CB	CYS	A	186	12.220	58.669	91.429	1.00 21.60
ATOM	1353	SG	CYS	A	186	12.075	58.576	93.237	1.00 25.16
ATOM	1354	N	GLY	A	187	11.149	61.494	89.814	1.00 20.34
ATOM	1355	CA	GLY	A	187	11.023	62.949	89.981	1.00 13.75
ATOM	1356	C	GLY	A	187	12.351	63.676	90.197	1.00 14.55
ATOM	1357	O	GLY	A	187	12.436	64.871	90.453	1.00 16.08
ATOM	1358	N	TYR	A	188	13.434	62.894	90.072	1.00 14.86
ATOM	1359	CA	TYR	A	188	14.736	63.526	90.226	1.00 16.85
ATOM	1360	C	TYR	A	188	15.214	64.366	89.046	1.00 20.24
ATOM	1361	O	TYR	A	188	15.979	65.318	89.192	1.00 18.42
ATOM	1362	CB	TYR	A	188	15.758	62.441	90.479	1.00 21.64
ATOM	1363	CG	TYR	A	188	15.683	61.913	91.899	1.00 27.20
ATOM	1364	CD1	TYR	A	188	16.748	62.226	92.737	1.00 32.00
ATOM	1365	CD2	TYR	A	188	14.604	61.128	92.365	1.00 30.81
ATOM	1366	CE1	TYR	A	188	16.727	61.784	94.071	1.00 35.38
ATOM	1367	CE2	TYR	A	188	14.570	60.695	93.705	1.00 30.65
ATOM	1368	CZ	TYR	A	188	15.624	61.071	94.556	1.00 35.88
ATOM	1369	OH	TYR	A	188	15.607	60.787	95.912	1.00 41.39
ATOM	1370	N	HIS	A	189	14.751	63.890	87.862	1.00 19.35
ATOM	1371	CA	HIS	A	189	15.215	64.475	86.589	1.00 19.83
ATOM	1372	C	HIS	A	189	14.108	64.550	85.551	1.00 16.85
ATOM	1373	O	HIS	A	189	13.232	63.689	85.533	1.00 18.10
ATOM	1374	CB	HIS	A	189	16.360	63.648	86.032	1.00 15.43
ATOM	1375	CG	HIS	A	189	17.677	64.142	86.589	1.00 16.05
ATOM	1376	ND1	HIS	A	189	18.154	65.384	86.414	1.00 17.24
ATOM	1377	CD2	HIS	A	189	18.581	63.433	87.382	1.00 17.58
ATOM	1378	CE1	HIS	A	189	19.347	65.503	87.080	1.00 16.38
ATOM	1379	NE2	HIS	A	189	19.587	64.304	87.667	1.00 18.68
ATOM	1380	N	TRP	A	190	14.183	65.611	84.723	1.00 18.99
ATOM	1381	CA	TRP	A	190	13.341	65.719	83.490	1.00 17.22
ATOM	1382	C	TRP	A	190	13.768	64.690	82.453	1.00 15.79
ATOM	1383	O	TRP	A	190	14.942	64.354	82.416	1.00 15.77
ATOM	1384	CB	TRP	A	190	13.502	67.102	82.855	1.00 16.12
ATOM	1385	CG	TRP	A	190	13.134	68.178	83.857	1.00 12.39
ATOM	1386	CD1	TRP	A	190	14.033	69.073	84.454	1.00 10.88
ATOM	1387	CD2	TRP	A	190	11.800	68.581	84.295	1.00 14.35
ATOM	1388	NE1	TRP	A	190	13.343	69.989	85.186	1.00 13.77
ATOM	1389	CE2	TRP	A	190	11.976	69.731	85.141	1.00 10.71
ATOM	1390	CE3	TRP	A	190	10.505	68.036	84.094	1.00 14.68
ATOM	1391	CZ2	TRP	A	190	10.845	70.401	85.666	1.00 12.89
ATOM	1392	CZ3	TRP	A	190	9.393	68.700	84.672	1.00 16.93
ATOM	1393	CH2	TRP	A	190	9.557	69.875	85.441	1.00 12.82
ATOM	1394	N	GLN	A	191	12.859	64.188	81.613	1.00 16.92
ATOM	1395	CA	GLN	A	191	13.316	63.234	80.569	1.00 17.49
ATOM	1396	C	GLN	A	191	14.519	63.720	79.696	1.00 15.64
ATOM	1397	O	GLN	A	191	15.508	63.054	79.429	1.00 16.52
ATOM	1398	CB	GLN	A	191	12.113	62.829	79.721	1.00 15.28
ATOM	1399	CG	GLN	A	191	12.522	61.832	78.632	1.00 17.26
ATOM	1400	CD	GLN	A	191	12.860	60.493	79.206	1.00 16.84
ATOM	1401	OE1	GLN	A	191	12.086	59.920	79.946	1.00 21.21
ATOM	1402	NE2	GLN	A	191	14.027	59.971	78.864	1.00 16.69
ATOM	1403	N	SER	A	192	14.448	65.016	79.389	1.00 17.04
ATOM	1404	CA	SER	A	192	15.564	65.622	78.666	1.00 15.81

[Drawing 36]

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ATOM   1405  C   SER A 192      16.899  65.683  79.339  1.00 17.61
ATOM   1406  O   SER A 192      17.937  65.640  78.700  1.00 16.65
ATOM   1407  CB  SER A 192      15.209  67.005  78.211  1.00 15.87
ATOM   1408  OG  SER A 192      14.862  67.833  79.313  1.00 21.12
ATOM   1409  N   ASP A 193      16.886  65.712  80.681  1.00 17.44
ATOM   1410  CA  ASP A 193      18.190  65.648  81.360  1.00 15.23
ATOM   1411  C   ASP A 193      18.889  64.316  81.197  1.00 12.26
ATOM   1412  O   ASP A 193      20.113  64.204  81.054  1.00 14.16
ATOM   1413  CB  ASP A 193      18.036  65.832  82.887  1.00 16.01
ATOM   1414  CG  ASP A 193      17.367  67.116  83.311  1.00 14.57
ATOM   1415  OD1 ASP A 193      17.503  68.180  82.691  1.00 15.89
ATOM   1416  OD2 ASP A 193      16.673  67.038  84.312  1.00 19.14
ATOM   1417  N   VAL A 194      18.025  63.283  81.216  1.00 11.48
ATOM   1418  CA  VAL A 194      18.425  61.882  81.108  1.00 13.18
ATOM   1419  C   VAL A 194      18.851  61.478  79.663  1.00 15.63
ATOM   1420  O   VAL A 194      19.852  60.822  79.387  1.00 15.79
ATOM   1421  CB  VAL A 194      17.210  61.135  81.625  1.00 16.33
ATOM   1422  CG1 VAL A 194      17.152  61.202  83.184  1.00 19.26
ATOM   1423  CG2 VAL A 194      17.079  59.712  81.106  1.00 17.70
ATOM   1424  N   ASP A 195      18.050  61.992  78.728  1.00 16.54
ATOM   1425  CA  ASP A 195      18.488  61.921  77.332  1.00 16.55
ATOM   1426  C   ASP A 195      19.801  62.636  77.029  1.00 14.74
ATOM   1427  O   ASP A 195      20.758  62.076  76.519  1.00 18.92
ATOM   1428  CB  ASP A 195      17.367  62.455  76.477  1.00 15.91
ATOM   1429  CG  ASP A 195      16.139  61.563  76.560  1.00 19.86
ATOM   1430  OD1 ASP A 195      16.153  60.385  76.922  1.00 27.62
ATOM   1431  OD2 ASP A 195      15.090  62.069  76.264  1.00 26.20
ATOM   1432  N   ALA A 196      19.902  63.900  77.450  1.00 15.85
ATOM   1433  CA  ALA A 196      21.203  64.555  77.312  1.00 14.73
ATOM   1434  C   ALA A 196      22.383  63.806  77.932  1.00 18.85
ATOM   1435  O   ALA A 196      23.512  63.751  77.429  1.00 21.06
ATOM   1436  CB  ALA A 196      21.134  65.950  77.904  1.00 13.59
ATOM   1437  N   ALA A 197      22.056  63.177  79.091  1.00 19.14
ATOM   1438  CA  ALA A 197      23.098  62.442  79.808  1.00 17.62
ATOM   1439  C   ALA A 197      23.644  61.202  79.090  1.00 17.71
ATOM   1440  O   ALA A 197      24.851  60.931  79.104  1.00 18.18
ATOM   1441  CB  ALA A 197      22.587  62.002  81.181  1.00 15.91
ATOM   1442  N   ARG A 198      22.711  60.477  78.418  1.00 16.04
ATOM   1443  CA  ARG A 198      23.238  59.409  77.565  1.00 15.41
ATOM   1444  C   ARG A 198      24.179  59.843  76.413  1.00 14.99
ATOM   1445  O   ARG A 198      25.194  59.219  76.113  1.00 17.07
ATOM   1446  CB  ARG A 198      22.136  58.469  77.080  1.00 14.50
ATOM   1447  CG  ARG A 198      21.195  58.043  78.179  1.00 16.67
ATOM   1448  CD  ARG A 198      20.142  57.044  77.730  1.00 19.20
ATOM   1449  NE  ARG A 198      19.280  56.629  78.849  1.00 22.72
ATOM   1450  CZ  ARG A 198      18.003  57.012  79.061  1.00 22.30
ATOM   1451  NH1 ARG A 198      17.412  57.905  78.325  1.00 20.95
ATOM   1452  NH2 ARG A 198      17.292  56.518  80.045  1.00 21.57
ATOM   1453  N   VAL A 199      23.907  61.030  75.842  1.00 17.69
ATOM   1454  CA  VAL A 199      24.961  61.510  74.913  1.00 17.44
ATOM   1455  C   VAL A 199      26.376  61.736  75.457  1.00 18.48
ATOM   1456  O   VAL A 199      27.360  61.145  74.988  1.00 19.25
ATOM   1457  CB  VAL A 199      24.452  62.820  74.284  1.00 15.93
ATOM   1458  CG1 VAL A 199      25.350  63.540  73.279  1.00 11.45
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[Drawing 37]

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ATOM   1459  CG2 VAL A 199      23.072  62.640  73.680  1.00 15.33
ATOM   1460  N   VAL A 200      26.471  62.548  76.550  1.00 18.52
ATOM   1461  CA  VAL A 200      27.822  62.723  77.153  1.00 16.27
ATOM   1462  C   VAL A 200      28.442  61.461  77.794  1.00 14.37
ATOM   1463  O   VAL A 200      29.643  61.247  77.797  1.00 17.25
ATOM   1464  CB  VAL A 200      27.811  63.938  78.100  1.00 16.56
ATOM   1465  CG1 VAL A 200      26.761  63.894  79.246  1.00 13.52
ATOM   1466  CG2 VAL A 200      27.666  65.177  77.226  1.00 17.14
ATOM   1467  N   GLY A 201      27.556  60.570  78.283  1.00 14.98
ATOM   1468  CA  GLY A 201      27.998  59.297  78.836  1.00 13.20
ATOM   1469  C   GLY A 201      28.609  58.377  77.824  1.00 16.81
ATOM   1470  O   GLY A 201      29.588  57.701  78.067  1.00 17.03
ATOM   1471  N   SER A 202      28.034  58.413  76.614  1.00 17.82
ATOM   1472  CA  SER A 202      28.757  57.750  75.509  1.00 16.70
ATOM   1473  C   SER A 202      30.087  58.390  75.104  1.00 13.91
ATOM   1474  O   SER A 202      31.117  57.765  74.939  1.00 18.47
ATOM   1475  CB  SER A 202      27.813  57.511  74.303  1.00 15.94
ATOM   1476  OG  SER A 202      27.634  58.737  73.600  1.00 19.26
ATOM   1477  N   ALA A 203      30.094  59.719  75.030  1.00 14.76
ATOM   1478  CA  ALA A 203      31.333  60.383  74.641  1.00 14.41
ATOM   1479  C   ALA A 203      32.527  60.110  75.563  1.00 17.92
ATOM   1480  O   ALA A 203      33.652  59.803  75.177  1.00 16.36
ATOM   1481  CB  ALA A 203      31.042  61.872  74.572  1.00 13.61
ATOM   1482  N   VAL A 204      32.200  60.126  76.880  1.00 18.27
ATOM   1483  CA  VAL A 204      33.290  59.835  77.823  1.00 16.45
ATOM   1484  C   VAL A 204      33.834  58.394  77.718  1.00 13.88
ATOM   1485  O   VAL A 204      35.015  58.177  77.916  1.00 17.64
ATOM   1486  CB  VAL A 204      32.893  60.245  79.285  1.00 16.63
ATOM   1487  CG1 VAL A 204      34.131  60.323  80.177  1.00 16.51
ATOM   1488  CG2 VAL A 204      31.836  59.301  79.862  1.00 13.53
ATOM   1489  N   VAL A 205      32.971  57.392  77.355  1.00 15.31
ATOM   1490  CA  VAL A 205      33.648  56.100  77.152  1.00 17.30
ATOM   1491  C   VAL A 205      34.636  56.018  75.953  1.00 19.20
ATOM   1492  O   VAL A 205      35.644  55.311  76.002  1.00 19.08
ATOM   1493  CB  VAL A 205      32.563  55.014  77.035  1.00 19.10
ATOM   1494  CG1 VAL A 205      32.812  53.549  76.681  1.00 16.51
ATOM   1495  CG2 VAL A 205      31.797  55.088  78.343  1.00 17.27
ATOM   1496  N   ALA A 206      34.409  56.893  74.934  1.00 21.03
ATOM   1497  CA  ALA A 206      35.452  57.018  73.888  1.00 19.17
ATOM   1498  C   ALA A 206      36.765  57.498  74.469  1.00 19.27
ATOM   1499  O   ALA A 206      37.809  56.868  74.353  1.00 18.59
ATOM   1500  CB  ALA A 206      34.982  57.988  72.809  1.00 16.60
ATOM   1501  N   THR A 207      36.643  58.597  75.234  1.00 20.20
ATOM   1502  CA  THR A 207      37.873  59.078  75.903  1.00 20.52
ATOM   1503  C   THR A 207      38.613  58.126  76.838  1.00 21.84
ATOM   1504  O   THR A 207      39.831  58.030  76.898  1.00 23.45
ATOM   1505  CB  THR A 207      37.659  60.341  76.674  1.00 19.84
ATOM   1506  OG1 THR A 207      36.577  61.100  76.137  1.00 20.11
ATOM   1507  CG2 THR A 207      38.945  61.141  76.710  1.00 21.15
ATOM   1508  N   LEU A 208      37.806  57.345  77.574  1.00 23.89
ATOM   1509  CA  LEU A 208      38.323  56.332  78.510  1.00 22.19
ATOM   1510  C   LEU A 208      39.165  55.285  77.783  1.00 25.28
ATOM   1511  O   LEU A 208      40.232  54.880  78.228  1.00 24.41
ATOM   1512  CB  LEU A 208      37.170  55.700  79.312  1.00 21.93
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[Drawing 3H]

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ATOM   1513  CG  LEU A 208      36.238  56.370  80.325  1.00 24.99
ATOM   1514  CD1 LEU A 208      35.117  55.631  81.058  1.00 26.55
ATOM   1515  CD2 LEU A 208      37.303  56.685  81.377  1.00 22.67
ATOM   1516  N   HIS A 209      38.666  54.873  76.588  1.00 24.64
ATOM   1517  CA  HIS A 209      39.495  53.970  75.766  1.00 20.81
ATOM   1518  C   HIS A 209      40.863  54.530  75.277  1.00 19.15
ATOM   1519  O   HIS A 209      41.807  53.872  74.934  1.00 20.30
ATOM   1520  CB  HIS A 209      38.656  53.449  74.595  1.00 16.87
ATOM   1521  CG  HIS A 209      37.588  52.476  74.994  1.00 13.42
ATOM   1522  ND1 HIS A 209      36.335  52.782  75.375  1.00 14.12
ATOM   1523  CD2 HIS A 209      37.686  51.099  74.975  1.00 12.37
ATOM   1524  CE1 HIS A 209      35.653  51.616  75.586  1.00 10.36
ATOM   1525  NE2 HIS A 209      36.493  50.590  75.334  1.00 13.53
ATOM   1526  N   THR A 210      41.035  55.827  75.336  1.00 20.82
ATOM   1527  CA  THR A 210      42.393  56.384  75.116  1.00 21.40
ATOM   1528  C   THR A 210      43.396  56.387  76.298  1.00 26.34
ATOM   1529  O   THR A 210      44.567  56.745  76.188  1.00 27.83
ATOM   1530  CB  THR A 210      42.315  57.836  74.662  1.00 22.02
ATOM   1531  OG1 THR A 210      42.096  58.723  75.795  1.00 25.04
ATOM   1532  CG2 THR A 210      41.307  58.070  73.528  1.00 20.46
ATOM   1533  N   ASN A 211      42.844  56.032  77.482  1.00 26.32
ATOM   1534  CA  ASN A 211      43.544  56.135  78.782  1.00 23.64
ATOM   1535  C   ASN A 211      44.212  54.824  79.204  1.00 20.62
ATOM   1536  O   ASN A 211      43.591  53.785  79.374  1.00 22.28
ATOM   1537  CB  ASN A 211      42.563  56.749  79.827  1.00 24.99
ATOM   1538  CG  ASN A 211      43.226  56.863  81.206  1.00 24.09
ATOM   1539  OD1 ASN A 211      43.320  55.886  81.935  1.00 25.25
ATOM   1540  ND2 ASN A 211      43.689  58.038  81.566  1.00 21.24
ATOM   1541  N   PRO A 212      45.563  54.884  79.353  1.00 22.01
ATOM   1542  CA  PRO A 212      46.337  53.660  79.633  1.00 21.52
ATOM   1543  C   PRO A 212      45.859  52.883  80.848  1.00 22.52
ATOM   1544  O   PRO A 212      45.670  51.673  80.882  1.00 22.12
ATOM   1545  CB  PRO A 212      47.743  54.190  79.845  1.00 22.52
ATOM   1546  CG  PRO A 212      47.805  55.535  79.117  1.00 25.40
ATOM   1547  CD  PRO A 212      46.391  56.076  79.175  1.00 22.01
ATOM   1548  N   ALA A 213      45.626  53.674  81.897  1.00 23.96
ATOM   1549  CA  ALA A 213      45.139  53.025  83.140  1.00 23.57
ATOM   1550  C   ALA A 213      43.797  52.337  83.019  1.00 21.76
ATOM   1551  O   ALA A 213      43.600  51.185  83.403  1.00 24.96
ATOM   1552  CB  ALA A 213      45.039  54.071  84.259  1.00 21.55
ATOM   1553  N   PHE A 214      42.885  53.085  82.373  1.00 19.30
ATOM   1554  CA  PHE A 214      41.617  52.431  82.017  1.00 20.02
ATOM   1555  C   PHE A 214      41.798  51.170  81.197  1.00 20.38
ATOM   1556  O   PHE A 214      41.255  50.120  81.510  1.00 18.82
ATOM   1557  CB  PHE A 214      40.690  53.445  81.314  1.00 23.58
ATOM   1558  CG  PHE A 214      39.367  52.839  80.840  1.00 26.35
ATOM   1559  CD1 PHE A 214      38.249  52.765  81.711  1.00 24.66
ATOM   1560  CD2 PHE A 214      39.262  52.365  79.507  1.00 23.08
ATOM   1561  CE1 PHE A 214      37.032  52.191  81.265  1.00 27.05
ATOM   1562  CE2 PHE A 214      38.052  51.792  79.077  1.00 19.74
ATOM   1563  CZ  PHE A 214      36.951  51.697  79.944  1.00 22.04
ATOM   1564  N   GLN A 215      42.654  51.298  80.149  1.00 22.02
ATOM   1565  CA  GLN A 215      42.904  50.137  79.275  1.00 21.33
ATOM   1566  C   GLN A 215      43.354  48.889  79.995  1.00 21.73
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[Drawing 30]

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ATOM   1567  O   GLN A 215      42.823  47.783  79.875  1.00 21.17
ATOM   1568  CB  GLN A 215      43.970  50.520  78.278  1.00 22.37
ATOM   1569  CG  GLN A 215      43.483  51.517  77.261  1.00 22.30
ATOM   1570  CD  GLN A 215      44.662  52.026  76.479  1.00 28.18
ATOM   1571  OE1 GLN A 215      45.821  51.695  76.664  1.00 32.27
ATOM   1572  NE2 GLN A 215      44.359  52.947  75.605  1.00 28.35
ATOM   1573  N   GLN A 216      44.375  49.154  80.845  1.00 24.60
ATOM   1574  CA  GLN A 216      44.876  48.089  81.718  1.00 25.81
ATOM   1575  C   GLN A 216      43.909  47.530  82.715  1.00 23.07
ATOM   1576  O   GLN A 216      43.822  46.328  82.899  1.00 22.91
ATOM   1577  CB  GLN A 216      46.052  48.544  82.517  1.00 33.78
ATOM   1578  CG  GLN A 216      47.181  49.037  81.631  1.00 49.94
ATOM   1579  CD  GLN A 216      48.161  49.693  82.574  1.00 61.57
ATOM   1580  OE1 GLN A 216      48.354  49.243  83.704  1.00 69.34
ATOM   1581  NE2 GLN A 216      48.737  50.805  82.114  1.00 63.50
ATOM   1582  N   GLN A 217      43.155  48.439  83.377  1.00 22.58
ATOM   1583  CA  GLN A 217      42.099  47.917  84.261  1.00 23.69
ATOM   1584  C   GLN A 217      40.971  47.113  83.590  1.00 24.92
ATOM   1585  O   GLN A 217      40.480  46.102  84.088  1.00 24.09
ATOM   1586  CB  GLN A 217      41.565  49.042  85.189  1.00 23.44
ATOM   1587  CG  GLN A 217      40.720  48.541  86.407  1.00 23.24
ATOM   1588  CD  GLN A 217      41.489  47.589  87.335  1.00 21.58
ATOM   1589  OE1 GLN A 217      42.676  47.749  87.598  1.00 24.82
ATOM   1590  NE2 GLN A 217      40.827  46.516  87.744  1.00 19.85
ATOM   1591  N   LEU A 218      40.628  47.595  82.390  1.00 26.00
ATOM   1592  CA  LEU A 218      39.701  46.859  81.532  1.00 23.47
ATOM   1593  C   LEU A 218      40.195  45.495  81.093  1.00 21.96
ATOM   1594  O   LEU A 218      39.476  44.515  81.209  1.00 22.51
ATOM   1595  CB  LEU A 218      39.309  47.724  80.327  1.00 23.65
ATOM   1596  CG  LEU A 218      38.292  47.073  79.369  1.00 21.04
ATOM   1597  CD1 LEU A 218      38.103  47.980  78.168  1.00 25.30
ATOM   1598  CD2 LEU A 218      36.952  46.736  80.004  1.00 13.22
ATOM   1599  N   GLN A 219      41.451  45.425  80.640  1.00 23.49
ATOM   1600  CA  GLN A 219      42.033  44.079  80.457  1.00 29.37
ATOM   1601  C   GLN A 219      41.880  43.156  81.681  1.00 29.60
ATOM   1602  O   GLN A 219      41.455  42.016  81.569  1.00 29.63
ATOM   1603  CB  GLN A 219      43.544  44.131  80.199  1.00 37.46
ATOM   1604  CG  GLN A 219      44.052  44.703  78.867  1.00 51.74
ATOM   1605  CD  GLN A 219      45.511  45.267  78.911  1.00 60.29
ATOM   1606  OE1 GLN A 219      46.415  44.774  79.568  1.00 65.45
ATOM   1607  NE2 GLN A 219      45.764  46.352  78.161  1.00 60.81
ATOM   1608  N   LYS A 220      42.206  43.722  82.879  1.00 28.28
ATOM   1609  CA  LYS A 220      42.004  42.926  84.111  1.00 26.68
ATOM   1610  C   LYS A 220      40.588  42.446  84.386  1.00 24.54
ATOM   1611  O   LYS A 220      40.347  41.275  84.640  1.00 26.87
ATOM   1612  CB  LYS A 220      42.591  43.631  85.319  1.00 29.93
ATOM   1613  CG  LYS A 220      44.019  43.952  84.934  1.00 36.96
ATOM   1614  CD  LYS A 220      45.015  44.044  86.081  1.00 47.54
ATOM   1615  CE  LYS A 220      44.741  45.121  87.121  1.00 55.28
ATOM   1616  NZ  LYS A 220      44.868  46.456  86.510  1.00 61.92
ATOM   1617  N   ALA A 221      39.630  43.379  84.217  1.00 21.04
ATOM   1618  CA  ALA A 221      38.215  42.960  84.307  1.00 18.69
ATOM   1619  C   ALA A 221      37.761  41.903  83.291  1.00 24.31
ATOM   1620  O   ALA A 221      37.095  40.921  83.598  1.00 26.78
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[Drawing 40]

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ATOM   1621  CB  ALA A 221      37.306  44.177  84.140  1.00 14.85
ATOM   1622  N   LYS A 222      38.223  42.106  82.029  1.00 24.15
ATOM   1623  CA  LYS A 222      38.065  41.018  81.046  1.00 23.96
ATOM   1624  C   LYS A 222      38.668  39.675  81.431  1.00 22.61
ATOM   1625  O   LYS A 222      38.023  38.628  81.422  1.00 21.88
ATOM   1626  CB  LYS A 222      38.591  41.444  79.659  1.00 22.92
ATOM   1627  CG  LYS A 222      37.682  42.516  79.109  1.00 22.76
ATOM   1628  CD  LYS A 222      38.038  42.903  77.691  1.00 22.50
ATOM   1629  CE  LYS A 222      37.050  43.918  77.109  1.00 22.07
ATOM   1630  NZ  LYS A 222      37.556  44.613  75.909  1.00 21.47
ATOM   1631  N   ALA A 223      39.949  39.728  81.830  1.00 22.84
ATOM   1632  CA  ALA A 223      40.533  38.472  82.353  1.00 25.17
ATOM   1633  C   ALA A 223      39.812  37.842  83.543  1.00 26.19
ATOM   1634  O   ALA A 223      39.534  36.652  83.573  1.00 27.99
ATOM   1635  CB  ALA A 223      42.013  38.638  82.691  1.00 21.36
ATOM   1636  N   GLU A 224      39.424  38.696  84.487  1.00 27.72
ATOM   1637  CA  GLU A 224      38.643  38.187  85.610  1.00 27.59
ATOM   1638  C   GLU A 224      37.338  37.525  85.191  1.00 28.89
ATOM   1639  O   GLU A 224      36.971  36.412  85.568  1.00 28.75
ATOM   1640  CB  GLU A 224      38.476  39.348  86.606  1.00 29.18
ATOM   1641  CG  GLU A 224      37.470  39.099  87.741  1.00 29.05
ATOM   1642  CD  GLU A 224      37.335  40.348  88.557  1.00 29.41
ATOM   1643  OE1 GLU A 224      36.506  41.189  88.269  1.00 28.08
ATOM   1644  OE2 GLU A 224      38.060  40.487  89.516  1.00 31.14
ATOM   1645  N   PHE A 225      36.659  38.233  84.288  1.00 29.33
ATOM   1646  CA  PHE A 225      35.398  37.699  83.770  1.00 28.70
ATOM   1647  C   PHE A 225      35.551  36.354  83.061  1.00 31.53
ATOM   1648  O   PHE A 225      34.802  35.399  83.222  1.00 29.17
ATOM   1649  CB  PHE A 225      34.786  38.756  82.858  1.00 26.48
ATOM   1650  CG  PHE A 225      33.449  38.281  82.361  1.00 25.90
ATOM   1651  CD1 PHE A 225      32.361  38.258  83.250  1.00 28.71
ATOM   1652  CD2 PHE A 225      33.317  37.854  81.022  1.00 28.64
ATOM   1653  CE1 PHE A 225      31.129  37.758  82.817  1.00 29.98
ATOM   1654  CE2 PHE A 225      32.074  37.365  80.564  1.00 28.53
ATOM   1655  CZ  PHE A 225      30.998  37.309  81.479  1.00 30.02
ATOM   1656  N   ALA A 226      36.635  36.308  82.289  1.00 31.91
ATOM   1657  CA  ALA A 226      36.996  35.055  81.643  1.00 36.17
ATOM   1658  C   ALA A 226      37.178  33.829  82.536  1.00 40.94
ATOM   1659  O   ALA A 226      36.704  32.735  82.271  1.00 41.75
ATOM   1660  CB  ALA A 226      38.284  35.261  80.877  1.00 33.81
ATOM   1661  N   GLN A 227      37.883  34.081  83.647  1.00 44.91
ATOM   1662  CA  GLN A 227      38.067  32.931  84.543  1.00 49.32
ATOM   1663  C   GLN A 227      36.782  32.579  85.233  1.00 51.62
ATOM   1664  O   GLN A 227      36.396  31.467  85.528  1.00 50.02
ATOM   1665  CB  GLN A 227      39.239  33.150  85.499  1.00 50.80
ATOM   1666  CG  GLN A 227      40.441  33.937  84.900  1.00 59.92
ATOM   1667  CD  GLN A 227      40.812  33.681  83.397  1.00 68.68
ATOM   1668  OE1 GLN A 227      40.799  32.592  82.834  1.00 73.97
ATOM   1669  NE2 GLN A 227      41.221  34.764  82.726  1.00 65.71
ATOM   1670  N   HIS A 228      36.041  33.669  85.411  1.00 58.91
ATOM   1671  CA  HIS A 228      34.687  33.501  85.925  1.00 65.53
ATOM   1672  C   HIS A 228      33.816  32.519  85.143  1.00 68.04
ATOM   1673  O   HIS A 228      33.014  31.764  85.662  1.00 67.46
ATOM   1674  CB  HIS A 228      34.091  34.903  86.045  1.00 68.11
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[Drawing 41]

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ATOM   1675  CG  HIS A 228      32.632  34.780  86.283  1.00 71.87
ATOM   1676  ND1 HIS A 228      31.729  34.968  85.313  1.00 74.51
ATOM   1677  CD2 HIS A 228      32.007  34.363  87.458  1.00 75.53
ATOM   1678  CE1 HIS A 228      30.511  34.660  85.852  1.00 78.68
ATOM   1679  NE2 HIS A 228      30.682  34.288  87.171  1.00 80.02
ATOM   1680  N   GLN A 229      34.061  32.545  83.846  1.00 74.45
ATOM   1681  CA  GLN A 229      33.306  31.659  82.963  1.00 81.06
ATOM   1682  C   GLN A 229      33.569  30.149  83.028  1.00 85.59
ATOM   1683  O   GLN A 229      33.123  29.427  82.135  1.00 86.52
ATOM   1684  CB  GLN A 229      33.477  32.181  81.530  1.00 81.47
ATOM   1685  CG  GLN A 229      33.002  33.631  81.335  1.00 81.55
ATOM   1686  CD  GLN A 229      31.488  33.690  81.352  1.00 83.81
ATOM   1687  OE1 GLN A 229      30.804  33.832  82.355  1.00 82.35
ATOM   1688  NE2 GLN A 229      30.950  33.588  80.141  1.00 86.80
ATOM   1689  N   LYS A 230      34.317  29.749  84.086  1.00 91.08
ATOM   1690  CA  LYS A 230      34.965  28.444  84.325  1.00 95.45
ATOM   1691  CB  LYS A 230      33.976  27.245  84.052  1.00 97.51
ATOM   1692  CG  LYS A 230      34.256  26.053  83.073  1.00 98.30
ATOM   1693  CD  LYS A 230      34.035  26.121  81.534  1.00 98.30
ATOM   1694  CE  LYS A 230      34.810  27.172  80.713  1.00100.00
ATOM   1695  NZ  LYS A 230      36.244  27.239  81.067  1.00100.00
ATOM   1696  C   LYS A 230      36.409  28.279  83.743  1.00 97.06
ATOM   1697  OCT1 LYS A 230      36.876  29.108  82.942  1.00 95.86
ATOM   1698  OCT2 LYS A 230      37.052  27.241  83.957  1.00 99.89
ATOM   1935  S   SO4 S 231      22.561  63.872  89.148  1.00 45.29
ATOM   1936  O1  SO4 S 231      21.748  62.858  88.279  1.00 50.45
ATOM   1937  O2  SO4 S 231      21.648  64.707  90.036  1.00 51.74
ATOM   1938  O3  SO4 S 231      23.551  63.095  90.035  1.00 49.75
ATOM   1939  O4  SO4 S 231      23.260  64.912  88.285  1.00 44.08
ATOM      1  O   HOH W 232      10.522  63.513  85.670  1.00 17.86
ATOM      2  O   HOH W 233      34.116  63.633  80.578  1.00 20.45
ATOM      3  O   HOH W 234       7.928  61.775  88.229  1.00 15.62
ATOM      4  O   HOH W 235      10.374  64.545  82.597  1.00 14.58
ATOM      5  O   HOH W 236      15.375  75.641  85.508  1.00 22.07
ATOM      6  O   HOH W 237      20.773  44.507  86.785  1.00 18.67
ATOM      7  O   HOH W 238      32.701  49.912  75.935  1.00 15.79
ATOM      8  O   HOH W 239      21.979  72.096  84.493  1.00 19.08
ATOM      9  O   HOH W 240      13.158  73.905  82.705  1.00 27.34
ATOM     10  O   HOH W 241      14.358  71.880  73.410  1.00 26.83
ATOM     11  O   HOH W 242       5.537  80.043  74.802  1.00 23.33
ATOM     12  O   HOH W 243      36.136  62.604  78.407  1.00 23.19
ATOM     13  O   HOH W 244      30.393  53.028  87.579  1.00 19.02
ATOM     14  O   HOH W 245      28.532  49.107  93.252  1.00 21.32
ATOM     15  O   HOH W 246      24.657  73.146  75.882  1.00 20.92
ATOM     16  O   HOH W 247      10.080  55.567  81.848  1.00 33.80
ATOM     17  O   HOH W 248      29.907  52.840  73.379  1.00 22.59
ATOM     18  O   HOH W 249      38.583  48.054  74.575  1.00 24.10
ATOM     19  O   HOH W 250      29.465  68.020  86.676  1.00 32.30
ATOM     20  O   HOH W 251      12.847  73.680  85.460  1.00 40.76
ATOM     21  O   HOH W 252       5.516  59.770  95.129  1.00 40.84
ATOM     22  O   HOH W 253      42.504  47.354  77.319  1.00 30.77
ATOM     23  O   HOH W 254      13.495  75.378  74.412  1.00 22.57
ATOM     24  O   HOH W 255      17.100  76.564  77.737  1.00 30.00
ATOM     25  O   HOH W 256      33.508  40.103 102.712  1.00 26.49
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[Drawing 42]

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ATOM     26  O   HOH W 257      20.825  55.648  81.278  1.00 20.11
ATOM     27  O   HOH W 258      19.730  61.701  89.970  1.00 23.10
ATOM     28  O   HOH W 259       4.363  74.520  80.720  1.00 33.74
ATOM     29  O   HOH W 260      31.490  42.656  98.480  1.00 34.19
ATOM     30  O   HOH W 261       6.696  75.130  78.477  1.00 15.66
ATOM     31  O   HOH W 262      10.667  67.023  75.103  1.00 38.86
ATOM     32  O   HOH W 263       8.252  64.433  92.307  1.00 23.15
ATOM     33  O   HOH W 264      41.924  51.223  74.247  1.00 30.19
ATOM     34  O   HOH W 265       1.437  67.705  89.398  1.00 39.48
ATOM     35  O   HOH W 266       4.055  66.946  91.467  1.00 29.22
ATOM     36  O   HOH W 267       3.092  69.112  84.950  1.00 25.58
ATOM     37  O   HOH W 268       9.537  59.065  79.795  1.00 30.90
ATOM     38  O   HOH W 269       9.306  83.197  79.638  1.00 44.19
ATOM     39  O   HOH W 270      34.786  41.166  75.522  1.00 32.98
ATOM     40  O   HOH W 271      28.084  37.193  84.163  1.00 30.43
ATOM     41  O   HOH W 272      40.742  49.227  76.024  1.00 21.82
ATOM     42  O   HOH W 273      35.074  40.712  85.668  1.00 29.87
ATOM     43  O   HOH W 274      30.318  45.526  96.384  1.00 35.57
ATOM     44  O   HOH W 275      31.493  69.162  80.850  1.00 19.51
ATOM     45  O   HOH W 276      42.914  61.700  76.016  1.00 28.69
ATOM     46  O   HOH W 277      34.422  64.714  92.625  1.00 38.81
ATOM     47  O   HOH W 278      13.405  78.374  80.916  1.00 25.22
ATOM     48  O   HOH W 279      44.634  57.811  84.433  1.00 31.73
ATOM     49  O   HOH W 280      44.303  60.992  82.740  1.00 28.14
ATOM     50  O   HOH W 281      32.596  51.432  73.247  1.00 22.63
ATOM     51  O   HOH W 282      22.182  40.126  75.125  1.00 27.50
ATOM     52  O   HOH W 283      18.482  55.362  89.100  1.00 21.25
ATOM     53  O   HOH W 284      36.960  42.360  74.192  1.00 28.88
ATOM     54  O   HOH W 285      35.881  48.845  94.047  1.00 26.90
ATOM     55  O   HOH W 286      26.212  59.698  94.760  1.00 23.37
ATOM     56  O   HOH W 287      29.246  44.303  73.369  1.00 40.38
ATOM     57  O   HOH W 288      27.356  35.947  80.422  1.00 31.74
ATOM     58  O   HOH W 289      40.482  45.029  76.766  1.00 30.88
ATOM     59  O   HOH W 290      24.864  58.724  91.112  1.00 25.30
ATOM     60  O   HOH W 291      28.560  61.547  91.755  1.00 39.37
ATOM     61  O   HOH W 292      27.888  63.113  90.252  1.00 40.28
ATOM     62  O   HOH W 293      31.069  41.023 103.435  1.00 38.13
ATOM     63  O   HOH W 294       5.144  47.860  86.978  1.00 37.63
ATOM     64  O   HOH W 295      29.373  52.425  90.409  1.00 21.69
ATOM     65  O   HOH W 296      41.571  51.401  87.864  1.00 31.72
ATOM     66  O   HOH W 297      35.633  56.807 101.396  1.00 42.27
ATOM     67  O   HOH W 298      35.257  40.157  78.063  1.00 30.17
ATOM     68  O   HOH W 299      33.734  71.189  79.910  1.00 32.64
ATOM     69  O   HOH W 300      17.659  69.593  75.158  1.00 46.73
ATOM     70  O   HOH W 301      17.005  72.932  72.774  1.00 33.93
ATOM     71  O   HOH W 302      15.769  48.059  85.107  1.00 24.21
ATOM     72  O   HOH W 303      15.023  64.697  75.333  1.00 39.99
ATOM     73  O   HOH W 304      13.546  67.305  74.469  1.00 38.11
ATOM     74  O   HOH W 305      30.044  75.863  82.738  1.00 29.02
ATOM     75  O   HOH W 306       5.253  66.383  98.323  1.00 61.09
ATOM     76  O   HOH W 307      25.914  72.829  89.073  1.00 48.08
ATOM     77  O   HOH W 308      38.474  67.620  76.050  1.00 32.88
ATOM     78  O   HOH W 309      34.101  41.534 100.215  1.00 39.54
ATOM     79  O   HOH W 310      29.974  37.419  76.650  1.00 39.99
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[Drawing 43]

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ATOM     80  O   HOH W 311      17.829  44.406  81.773  1.00 29.29
ATOM     81  O   HOH W 312      17.766  66.478  75.705  1.00 34.51
ATOM     82  O   HOH W 313      35.983  70.225  78.152  1.00 38.85
ATOM     83  O   HOH W 314      18.063  58.644  75.592  1.00 33.24
ATOM     84  O   HOH W 315      17.740  45.824  75.692  1.00 31.29
ATOM     85  O   HOH W 316      21.442  55.668 101.498  1.00 30.06
ATOM     86  O   HOH W 317      30.660  37.639 105.501  1.00 46.34
ATOM     87  O   HOH W 318      28.143  47.582  99.410  1.00 71.00
ATOM     88  O   HOH W 319      11.398  65.394  76.821  1.00 34.86
ATOM     89  O   HOH W 320      31.737  45.760  98.744  1.00 38.11
ATOM     90  O   HOH W 321      16.084  45.559  87.137  1.00 43.68
ATOM     91  O   HOH W 322      36.498  37.962  78.989  1.00 35.45
ATOM     92  O   HOH W 323      41.868  42.172  76.980  1.00 56.04
ATOM     93  O   HOH W 324      44.704  68.004  76.606  1.00 73.28
ATOM     94  O   HOH W 325      30.214  44.935 101.119  1.00 28.63
ATOM     95  O   HOH W 326      43.719  69.244  83.004  1.00 32.20
ATOM     96  O   HOH W 327       7.992  54.768  93.490  1.00 36.05
ATOM     97  O   HOH W 328      11.059  49.604  75.476  1.00 43.80
ATOM     98  O   HOH W 329      17.730  37.202  79.516  1.00 44.41
ATOM     99  O   HOH W 330      14.170  59.796  74.913  1.00 70.26
ATOM    100  O   HOH W 331      28.648  70.326  88.645  1.00 34.35
ATOM    101  O   HOH W 332      16.146  57.197  73.492  1.00 49.27
ATOM    102  O   HOH W 333      11.086  52.502  82.116  1.00 39.47
ATOM    103  O   HOH W 334      15.950  60.744  73.392  1.00 63.16
ATOM    104  O   HOH W 335      23.809  74.443  89.142  1.00 63.73
ATOM    105  O   HOH W 336      43.077  70.945  86.543  1.00 41.77
ATOM    106  O   HOH W 337      44.625  68.578  85.466  1.00 42.53
ATOM    107  O   HOH W 338      38.003  70.941  79.707  1.00 47.97
ATOM    108  O   HOH W 339      42.635  39.826  86.317  1.00 39.90
ATOM    109  O   HOH W 340      28.158  51.028  97.893  1.00 35.28
ATOM    110  O   HOH W 341      34.562  57.666  98.193  1.00 56.42
ATOM    111  O   HOH W 342      23.659  34.535  79.197  1.00 84.39
ATOM    112  O   HOH W 343      10.337  58.458  76.704  1.00 45.85
ATOM    113  O   HOH W 344      32.164  75.101  85.461  1.00 54.21
ATOM    114  O   HOH W 345      32.930  38.410  86.586  1.00 43.15
ATOM    115  O   HOH W 346      32.310  36.987 102.558  1.00 47.71
ATOM    116  O   HOH W 347      11.163  49.101  82.634  1.00 84.37
ATOM    117  O   HOH W 348      34.268  69.634  83.019  1.00 47.39
ATOM    118  O   HOH W 349      31.352  37.085  89.579  1.00 74.88
ATOM    119  O   HOH W 350      29.118  56.986  95.860  1.00 34.59
ATOM    120  O   HOH W 351       1.634  70.786  81.659  1.00 41.89
ATOM    121  O   HOH W 352       2.044  71.714  85.736  1.00 37.84
ATOM    122  O   HOH W 353      16.219  75.511  74.471  1.00 44.53
ATOM    123  O   HOH W 354      24.035  45.705  97.204  1.00 48.11
ATOM    124  O   HOH W 355      17.939  77.382  82.853  1.00 65.65
ATOM    125  O   HOH W 356      12.504  76.991  70.634  1.00 50.43
ATOM    126  O   HOH W 357      16.951  78.295  74.889  1.00 47.02
ATOM    127  O   HOH W 358      15.777  75.404  81.566  1.00 33.68
ATOM    128  O   HOH W 359      37.401  72.376  82.831  1.00 50.52
ATOM    129  O   HOH W 360      14.060  44.359  88.918  1.00 80.84
ATOM    130  O   HOH W 361      32.619  76.123  75.757  1.00 42.84
ATOM    131  O   HOH W 362      21.836  66.226  94.339  1.00 63.40
ATOM    132  O   HOH W 363      16.011  46.526  82.837  1.00 38.42
ATOM    133  O   HOH W 364       7.716  57.886  82.470  1.00 50.22
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[Drawing 44]

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ATOM    134  O   HOH W 365      41.813  72.155  81.960  1.00 76.60
ATOM    135  O   HOH W 366       5.810  63.614  94.440  1.00 41.72
ATOM    136  O   HOH W 367      22.833  66.006  98.308  1.00 65.79
ATOM    137  O   HOH W 368      21.384  36.791  76.692  1.00 59.29
ATOM    138  O   HOH W 369      38.765  52.950  92.219  1.00 27.87
ATOM    139  O   HOH W 370      46.430  68.991  81.609  1.00 70.52
ATOM    140  O   HOH W 371      36.973  69.709  83.153  1.00 33.81
ATOM    141  O   HOH W 372      22.238  43.716  92.825  1.00 44.49
ATOM    142  O   HOH W 373      23.096  80.189  77.308  1.00 50.73
ATOM    143  O   HOH W 374       2.790  53.932  81.478  1.00 50.22
ATOM    144  O   HOH W 375       3.292  64.768  94.055  1.00 46.64
ATOM    145  O   HOH W 376      26.937  79.257  75.755  1.00 47.21
ATOM    146  O   HOH W 377      45.046  50.594  85.873  1.00 46.10
ATOM    147  O   HOH W 378      24.988  68.312  90.158  1.00 39.03
ATOM    148  O   HOH W 379       2.045  61.203  93.643  1.00 49.73
ATOM    149  O   HOH W 380      44.273  56.110  87.700  1.00 46.74
ATOM    150  O   HOH W 381      26.747  76.462  73.043  1.00 50.91
ATOM    151  O   HOH W 382      40.545  70.889  76.918  1.00 68.80
ATOM    152  O   HOH W 383      25.523  80.486  83.807  1.00 67.90
ATOM    153  O   HOH W 384      40.972  36.296  87.372  1.00 66.49
ATOM    154  O   HOH W 385      12.617  56.710  77.567  1.00 44.81
ATOM    155  O   HOH W 386      44.460  48.054  74.082  1.00 41.02
ATOM    156  O   HOH W 387      35.781  73.896  86.117  1.00 38.14
ATOM    157  O   HOH W 388      21.625  80.398  81.815  1.00 47.96
ATOM    158  O   HOH W 389      46.628  56.635  82.977  1.00 50.89
ATOM    159  O   HOH W 390      12.308  51.573  78.083  1.00 64.92
ATOM    160  O   HOH W 391      30.773  39.420  87.798  1.00 55.92
ATOM    161  O   HOH W 392      26.088  65.110  89.923  1.00 44.80
ATOM    162  O   HOH W 393      10.719  70.886  96.928  1.00 48.46
ATOM    163  O   HOH W 394      12.474  47.243  84.457  1.00 53.08
ATOM    164  O   HOH W 395      24.296  71.312  91.828  1.00 48.39
ATOM    165  O   HOH W 396       6.459  50.108  83.133  1.00 57.82
ATOM    166  O   HOH W 397      42.423  66.213  75.196  1.00 34.97
ATOM    167  O   HOH W 398      29.045  53.518 101.769  1.00 46.59
ATOM    168  O   HOH W 399      27.195  39.655 105.406  1.00 41.59
ATOM    169  O   HOH W 400       6.834  56.385  96.211  1.00 45.81
ATOM    170  O   HOH W 401      47.957  50.138  78.280  1.00 43.60
ATOM    171  O   HOH W 402      23.330  36.461  72.787  1.00 54.41
ATOM    172  O   HOH W 403      29.051  79.533  81.900  1.00 78.87
ATOM    173  O   HOH W 404      46.670  55.026  74.340  1.00 68.61
ATOM    174  O   HOH W 405      28.985  78.746  85.840  1.00 75.16
ATOM    175  O   HOH W 406      32.117  68.589  73.365  1.00 42.10
ATOM    176  O   HOH W 407      48.677  52.842  75.727  1.00 66.77
ATOM    177  O   HOH W 408      29.185  36.245  72.017  1.00 75.24
ATOM    178  O   HOH W 409      37.168  67.596  97.670  1.00 38.24
ATOM    179  O   HOH W 410      11.986  77.352  92.370  1.00 35.94
ATOM    180  O   HOH W 411      39.548  63.174  98.280  1.00 39.58
ATOM    181  O   HOH W 412      30.500  79.967  79.292  1.00 62.87
ATOM    182  O   HOH W 413      18.003  41.205  83.764  1.00 64.48
ATOM    183  O   HOH W 414      34.455  37.242  89.080  1.00 52.58
ATOM    184  O   HOH W 415      47.074  60.938  83.746  1.00 66.98
ATOM    185  O   HOH W 416      10.880  54.535  78.559  1.00 45.29
ATOM    186  O   HOH W 417      30.230  76.830  74.341  1.00 71.09
ATOM    187  O   HOH W 418      12.118  81.147  79.341  1.00 49.66
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[Drawing 45]

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ATOM    188  O   HOH W 419      32.095  76.945  80.548  1.00 63.23
ATOM    189  O   HOH W 420      -0.301  68.264  84.539  1.00 48.83
ATOM    190  O   HOH W 421      10.822  64.227 102.313  1.00 81.32
ATOM    191  O   HOH W 422      23.374  42.555 101.170  1.00 42.91
ATOM    192  O   HOH W 423      20.016  59.713  74.793  1.00 38.45
ATOM    193  O   HOH W 424      15.833  78.412  79.495  1.00 56.01
ATOM    194  O   HOH W 425      43.534  35.850  84.957  1.00 63.16
ATOM    195  O   HOH W 426      11.933  68.018  98.874  1.00 52.15
ATOM    196  O   HOH W 427      20.777  37.368  85.962  1.00 57.71
ATOM    197  O   HOH W 428      22.392  36.632  89.560  1.00 68.43
ATOM    198  O   HOH W 429      29.340  37.487 101.980  1.00 74.20
ATOM    199  O   HOH W 430      23.237  39.294  91.878  1.00 74.07
ATOM    200  O   HOH W 431      13.654  75.325  94.697  1.00 73.83
ATOM    201  O   HOH W 432      27.904  38.307  96.631  1.00 57.14
ATOM    202  O   HOH W 433      44.213  59.909  79.188  1.00 37.71
ATOM    203  O   HOH W 434       2.129  75.408  79.755  1.00 64.17
ATOM    204  O   HOH W 435      13.993  43.469  84.483  1.00 59.78
ATOM    205  O   HOH W 436      31.644  55.529  99.951  1.00 58.81
ATOM    206  O   HOH W 437       9.462  82.415  76.470  1.00 48.44
ATOM    207  O   HOH W 438      21.813  58.761  98.061  1.00 60.37
ATOM    208  O   HOH W 439      22.202  59.533  93.382  1.00 43.39
ATOM    209  O   HOH W 440      18.118  43.497  86.455  1.00 46.86
ATOM    210  O   HOH W 441      13.762  54.340 105.466  1.00 57.78
ATOM    211  O   HOH W 442      33.277  73.931  83.853  1.00 56.73
ATOM    212  O   HOH W 443      34.442  68.648  90.744  1.00 27.90
ATOM    213  O   HOH W 444      30.640  67.899  91.831  1.00 53.48
ATOM    214  O   HOH W 445      40.813  44.217  74.058  1.00 53.35
ATOM    215  O   HOH W 446      33.012  71.334  90.213  1.00 53.98
ATOM    216  O   HOH W 447      25.130  57.928 101.293  1.00 38.97
ATOM    217  O   HOH W 448       7.584  82.067  74.163  1.00 26.55
ATOM    218  O   HOH W 449      42.214  40.521  78.980  1.00 37.75
ATOM    219  O   HOH W 450       8.915  57.776 101.115  1.00 50.37
ATOM    220  O   HOH W 451      15.963  42.582  79.699  1.00 71.24
ATOM    221  O   HOH W 452      23.011  77.967  75.363  1.00 64.04
ATOM    222  O   HOH W 453      36.910  35.452  88.469  1.00 70.47
ATOM    223  O   HOH W 454      37.814  55.271  99.966  1.00 54.36
ATOM    224  O   HOH W 455      26.721  58.439  99.230  1.00 86.46
ATOM    225  O   HOH W 456      16.108  40.093  81.126  1.00 98.28
ATOM    226  O   HOH W 457      27.800  35.543  96.536  1.00 63.56
ATOM    227  O   HOH W 458       5.859  51.318  95.801  1.00 68.96
ATOM    228  O   HOH W 459       7.841  51.875  96.622  1.00 64.76
ATOM    229  O   HOH W 460      28.280  66.535  89.122  1.00 73.27
ATOM    230  O   HOH W 461      13.943  46.268  81.680  1.00 56.20
ATOM    231  O   HOH W 462      14.681  69.220  73.344  1.00 83.46
ATOM    232  O   HOH W 463      30.388  71.379  89.815  1.00 60.45
ATOM    233  O   HOH W 464       8.062  56.915  75.809  1.00 59.76
ATOM    234  O   HOH W 465      30.104  41.907 101.688  1.00 61.89
ATOM    235  O   HOH W 466       4.988  49.407  95.471  1.00 61.48
ATOM    236  O   HOH W 467       8.747  53.997  77.187  1.00 79.74
END
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[Drawing 46]

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A72F(s)    5'-CA-GAC-CTG-GCC-TTT-GGC-GAT-GTG-GC-3'
A72F(as)   3'-GT-CTG-GAC-CGG-AAA-CCG-CTA-CAC-CG-5'
                    D   L   A   F72 G   D   V
A72E(s)    5'-CA-GAC-CTG-GCC-GAA-GGC-GAT-GTG-GC-3'
A72E(as)   3'-GT-CTG-GAC-CGG-CTT-CCG-CTA-CAC-CG-5'
                    D   L   A   E72 G   D   V
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[Drawing 47]

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I103D(s)   5'-TG-ACC-AAT-ATG-GAC-GAG-GAC-GCC-GG-3'
I103D(as)  3'-AC-TGG-TTA-TAC-CTG-CTC-CTG-CGG-CC-5'
                  T    N   M   D103 E    D    A
T153N(s)   5'-GG-CAT-ACC-TCT-AAC-GGC-TGG-GCT-AC-3'
T153N(as)  3'-CC-GTA-TGG-AGA-TTG-CCG-ACC-CGA-TG-5'
                  H    T   S   N153 G    W    A
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[Drawing 48]

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L140F(s)   5'-AC-CAG-GAC-AAA-TTC-TCC-AAA-AAT-GG-3'
L140F(as)  3'-TG-GTC-CTG-TTT-AAG-AGG-TTT-TTA-CC-5'
                  Q    D   K   F140 S    K    N
L140K(s)   5'-AC-CAG-GAC-AAA-AAA-TCC-AAA-AAT-GG-3'
L140K(as)  3'-TG-GTC-CTG-TTT-TTT-AGG-TTT-TTA-CC-5'
                  Q    D   K   K140 S    K    N
L140E(s)   5'-AC-CAG-GAC-AAA-GAA-TCC-AAA-AAT-GG-3'
L140E(as)  3'-TG-GTC-CTG-TTT-CTT-AGG-TTT-TTA-CC-5'
                  Q    D   K   E140 S    K    N
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[Translation done.]

WRITTEN AMENDMENT

---------- [Written amendment]
[Filing date]September 14, Heisei 12 (2000.9.14)
[Amendment 1]
[Document to be Amended]Specification
[Item(s) to be Amended]0050
[Method of Amendment]Change
[Proposed Amendment]
[0050]Example 20 Enterobacter aerogenes IFO 12010 Refining of origin wild type acid phosphatase and determination of N-terminal-amino-acid arrangement
Refine acid phosphatase of the Enterobacter aerogenes IFO12010 origin from the culture object of Escherichia coli JM109/pENP110 of example 24 statement of JP,10-201481,A, and N-terminal-amino-acid arrangement is determined, The amino acid sequence of maturation protein was determined. Escherichia coli JM109/pENP110 are the bacillus which introduced the acid phosphatase gene of the Enterobacter aerogenes IFO12010 origin into 109 shares of Escherichia coli JM, and produce this acid phosphatase. The amino acid sequence of the precursor protein expected from the base sequence of this acid phosphatase gene is equivalent to the arrangement shown in the array number 10 of an array table. The amino acid sequence shown in the array number 10 is an amino acid sequence of L61 Q/A63 Q/E64 A/N67 D/S69 A/G72 D/T133 K/E134 D/I151T variant EA-AP. 50 ml of nutrient media (pH 7.0) containing 1 g/dl of peptone, 0.5 g/dl of yeast extracts, and 1 g/dl of salt were put into a 500-ml Sakaguchi flask, and it heat-sterilized for 20 minutes at 120 **. One platinum loop of Escherichia coli JM109/pENP110 was inoculated into this, and shaking culture was carried out at 30 ** for 16 hours. The biomass which collected biomasses from culture medium by centrifugal separation was suspended to the 100mM potassium phosphate buffer (pH 7.0) of 100 ml, ultrasonication was performed for 20 minutes at 4 **, and the biomass was crushed. The treating solution was centrifuged and the cell-free extract was prepared except for the insoluble fraction. Ammonium sulfate was added so that it might become this cell-free extract with saturation 30%. After removing the precipitate generated by centrifugal separation, additional addition of the ammonium sulfate was carried out so that it might become digestive liquor with saturation 60%. Centrifugal separation recovered the generated precipitate and it dissolved in the 100mM potassium phosphate buffer. After dialyzing this crude enzyme liquid 3 times to 500 ml of 100mM potassium phosphate buffers (pH 7.0), It charged in DEAE-TOYOPARU 650M column (phi3.0x10.0cm) equilibrated with 20mM potassium phosphate buffer (pH 7.0), and 20mM potassium phosphate buffer (pH 7.0) washed. Since phosphate transfer activity suited the bypassing fraction, the fractions concerned were collected. Ammonium sulfate was added so that it might become this activity fraction with saturation 35%, and this was made to stick to the butyl-Toyopearl column (phi3.0x7.0cm) equilibrated with 20mM potassium phosphate buffer (pH 7.0) which contains saturated ammonium sulfate 35%. This was eluted by the linear concentration gradient of the saturated potassium phosphate buffer (pH 7.0) 20% from saturation 35%. CM-TOYOPARU equilibrated with 10mM potassium phosphate buffer (pH 6.0) after collecting activity fractions and dialyzing to 10mM potassium phosphate buffer (pH 6.0) 1L It was made to stick to a column (phi3.0x7.0cm). This was eluted by the linear concentration gradient of the

potassium phosphate buffer (pH 6.0) containing 300mM potassium chloride from 0mM. These activity fractions were collected. The above operation refined the enzyme in which phosphate transfer activity is shown about 5 times with the recovery rate of about 16% more nearly eventually than a cell-free extract. This enzyme preparation was uniform in SDS-polyacrylamide electrophoresis. It is DITC about these refining enzymes. Membrane It is made to stick to [a milli gene / bio-search (Milligen/Biosearch) company make], When the amino acid sequence of the amino terminal was determined using Prosequencer 6625 (a milli gene / bio-search company make), the amino acid sequence of the amino terminal of 5 residue shown in the array number 98 of the array table was determined. Since the amino terminal of refining enzymes was started from the 21st alanine residue of the arrangement of the array number 10 of an array table, The amino acid sequence shown in the array number 10 of an array table is the arrangement of a precursor protein, and it was thought that peptide to the 20th phenylalanine residue was removed from the 1st methionine residue after translation. From this result, the amino acid sequence of mature-bodies protein is equivalent to the arrangement shown in the amino acid numbers 1-228 during the arrangement shown in the array number 10 of an array table.

[Amendment 2]

[Document to be Amended]Specification

[Item(s) to be Amended]0062

[Method of Amendment]Change

[Proposed Amendment]

[0062]Example 23 Enterobacter aerogenes whose compatibility over guanosine improved IFO 12010 Phosphorylation of the guanosine by an origin new variant acid phosphatase transgenics bacillus Each variant acid phosphatase gene. The included plasmid. introduced Escherichia coli . JM109/pENP180, Escherichia coli JM109/pENP320, Escherichia coli JM109/pENP340, Escherichia coli JM109/pENP410, Escherichia coli JM109/pENP510, And Escherichia coli JM109/pENP520 was inoculated into 50 ml of L culture media containing ampicillin 100microg/ml and IPTG1mM, and it cultivated at 37 ** for 16 hours. 10 g/dl of pyrophoric acid and 6.6 g/dl of grinding guanosine prepared like Example 1 of the Japanese-Patent-Application-No. No. 189226 [12 to] specification are dissolved in a 100mM acetic acid buffer (pH 4.5), It was made to react at 35 ** for 12 hours, maintaining [to this, add each biomass so that it may become 100 mg/dl by dry cell weight, and] pH to 4.5. The quantity of generated 5'-guanylic acid was shown in Table 17. Escherichia coli each whose bacillus which introduced the enzyme variant as shown in Table 17 is an old stock Productivity improved compared with JM109/pENP180, and generation accumulation of the 5'-guanylic acid was carried out with high yield.

[Translation done.]

(19)日本国特許庁（ＪＰ）　(12)公開特許公報（Ａ）　(11)特許出願公開番号
特開2001－136984
（P2001－136984A）
(43)公開日　平成13年５月22日(2001.5.22)

(51)Int.Cl.[7]	識別記号	ＦＩ		テーマコード（参考）
Ｃ１２Ｎ　15/09	ＺＮＡ	Ｃ１２Ｎ	1/15	
1/15			1/19	
1/19			1/21	
1/21			9/12	
5/10			9/16	Ｂ

審査請求　未請求　請求項の数17　ＯＬ　（全 98 頁）　最終頁に続く

(21)出願番号　特願2000－262120(P2000－262120)

(22)出願日　平成12年８月31日(2000.8.31)

(31)優先権主張番号　特願平11－249545
(32)優先日　平成11年９月３日(1999.9.3)
(33)優先権主張国　日本（ＪＰ）
特許法第30条第１項適用申請有り

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(54)【発明の名称】　変異型ヌクレオシド－５’－リン酸生産酵素

(57)【要約】　（修正有）
【課題】　ヌクレオシド－５’－リン酸生産能が向上した新規な変異型ヌクレオシド－５’－リン酸生産酵素、その取得のための新規な手段、及び該酵素の用途を提供する。
【解決手段】　ヌクレオシド－５’－リン酸生産酵素において、Lys残基１つ、Arg残基２つ、His残基２つが存在し、これらのＣα間距離が特定の範囲内にあり、かつその付近にヌクレオシドが結合するスペースを有する、リン酸基転移活性及び／又はホスファターゼ活性を有する酵素のヌクレオシド－５’－リン酸生産能が向上した変異型ヌクレオシド－５’－リン酸生産酵素。既知の酵素の結晶のＸ線構造解析に基づく変異の確定による該酵素の製造方法。
【効果】　より活性の高い酵素の取得が容易となった。

【特許請求の範囲】

【請求項１】　ヌクレオシドー５′ーリン酸生産酵素において、Lys残基１つ、Arg残基２つ、His残基２つが存在し、これらのＣα間距離が図１に示す範囲内にあり、かつその付近にヌクレオシドが結合するスペースを有する、リン酸基転移活性及び／又はホスファターゼ活性を有する酵素のヌクレオシドー５′ーリン酸生産能が向上した変異型ヌクレオシドー５′ーリン酸生産酵素。

【請求項２】　酵素の由来がエシェリヒア属細菌、モルガネラ属細菌、プロビデンシア属細菌、エンテロバクター属細菌、クレブジエラ属細菌、又はエンテロバクター属細菌に属するものである請求項１に記載の変異型ヌクレオシドー５′ーリン酸生産酵素。

【請求項３】　エシェリヒア・ブラッタエ由来酸性ホスファターゼの結晶をＸ線結晶構造解析して得られる原子座標データに示されている構造座標を元にして、イノシン、グアノシン等のヌクレオシド並びにそのリン酸化合物との結合様式を推定し、アミノ酸残基及び／又は補欠因子等の置換、追加、削除によりヌクレオシドー５′ーリン酸生産能が向上した請求項１記載の変異型ヌクレオシドー５′ーリン酸生産酵素。

【請求項４】　エシェリヒア・ブラッタエ由来酸性ホスファターゼのアミノ酸配列の以下の位置（エシェリヒア・ブラッタエ酸性ホスファターゼのSer７２、又はSer７２から１０Å以内にある残基）：１６、６７－７６、７８－７９、９６、９９－１００、１０２－１０４、１０６－１０８、１１５、１４０、１４８－１５４、１５７、１７９、１８３の少なくとも一つの位置に変化が生じている変異型ヌクレオシドー５′ーリン酸生産酵素。

【請求項５】　リン酸基転移活性及び／又はホスファターゼ活性を有する酵素で、エシェリヒア・ブラッタエ由来酸性ホスファターゼとのアミノ酸配列アラインメントをしたときに、エシェリヒア・ブラッタエ由来酸性ホスファターゼのアミノ酸配列の以下の位置（エシェリヒア・ブラッタエ酸性ホスファターゼのSer７２、又はSer７２から１０Å以内にある残基）：１６、６７－７６、７８－７９、９６、９９－１００、１０２－１０４、１０６－１０８、１１５、１４０、１４８－１５４、１５７、１７９、１８３に対応する少なくとも一つの位置に変化が生じている変異型ヌクレオシドー５′ーリン酸生産酵素。

【請求項６】　リン酸基転移活性及び／又はホスファターゼ活性を有する酵素で、エシェリヒア・ブラッタエ由来酸性ホスファターゼの３次元構造とのアラインメントをトレッディング法により行ったときに、エシェリヒア・ブラッタエ由来酸性ホスファターゼのアミノ酸配列の以下の位置（エシェリヒア・ブラッタエ酸性ホスファターゼのSer７２、又はSer７２から１０Å以内にある残基）：１６、６７－７６、７８－７９、９６、９９－１００、１０２－１０４、１０６－１０８、１１５、１４０、１４８－１５４、１５７、１７９、１８３に対応する少なくとも一つの位置に変化が生じている変異型ヌクレオシドー５′ーリン酸生産酵素。

【請求項７】　酵素の由来がエンテロバクター・アェロゲネス由来であり、そのアミノ酸配列において１４番目のロイシン残基、６１番目のロイシン残基、６３番目のアラニン残基、６４番目のグルタミン酸残基、６７番目のアスパラギン残基、６９番目のセリン残基、７０番目のアラニン残基、７１番目のグリシン残基、７２番目のグリシン残基、１０１番目のイソロイシン残基、１０２番目のグルタミン酸残基、１３３番目のスレオニン残基、１３４番目のグルタミン酸残基、１３８番目のロイシン残基、１４９番目のスレオニン残基、１５１番目のイソロイシン残基のうち少なくとも一つのアミノ酸残基が他のアミノ酸残基に置換されたものである変異型ヌクレオシドー５′ーリン酸生産酵素。

【請求項８】　リン酸基転移活性及び／又はホスファターゼ活性を有する酵素、あるいはそれとモリブデン酸との複合体の結晶をＸ線結晶構造解析して得られる立体構造から確定された、該酵素の活性部位、及び／又はそれから１０Å以内にあるアミノ酸残基を、置換、追加、削除をすることにより、ヌクレオシドー５′ーリン酸生産能が向上した変異型酵素を製造することを特徴とする変異型ヌクレオシドー５′ーリン酸生産酵素の製造方法。

【請求項９】　エシェリヒア・ブラッタエ由来酸性ホスファターゼの構造座標を使用して、ホスファターゼ又はリン酸基転移酵素の阻害剤を製造する方法。

【請求項１０】　リン酸基転移活性及び／又はホスファターゼ活性を有する酵素、あるいはそれとモリブデン酸との複合体のいずれかの結晶。

【請求項１１】　六方晶系の空間群Ｐ$6_3$２２を有する、エシェリヒア・ブラッタエ由来酸性ホスファターゼの結晶。

【請求項１２】　斜方晶系の空間群Ｐ$2_12_12_1$を有する、エシェリヒア・ブラッタエ由来酸性ホスファターゼＧ７４Ｄ／Ｉ１５３Ｔ変異型酵素の結晶。

【請求項１３】　三方晶系の空間群Ｐ3_121を有する、エシェリヒア・ブラッタエ由来酸性ホスファターゼとモリブデン酸との複合体（反応中間体アナログ）の結晶。

【請求項１４】　請求項１～７のいずれか１項に記載の酵素をコードする遺伝子。

【請求項１５】　請求項１４に記載の遺伝子を含む組換えＤＮＡ。

【請求項１６】　請求項１５に記載の組換えＤＮＡを保有する微生物。

【請求項１７】　請求項１～７のいずれか１項に記載の酵素、又はそれを含有する微生物、あるいは請求項１６に記載の微生物を、ヌクレオシド並びにリン酸供与体に作用させてヌクレオシドー５′ーリン酸を生成させ、こ

れを採取することを特徴とするヌクレオシドー５′ーリン酸の製造方法。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、ヌクレオシドー５′ーリン酸生産能が向上した変異型ヌクレオシドー５′ーリン酸生産酵素、及びその製造方法に関する。また、本発明は、上記した酵素の製造に有用な酵素類に関する。更に本発明は、ヌクレオシドー５′ーリン酸の製造方法に関し、またその製造方法に有用な、前記の変異型酵素をコードする遺伝子、該遺伝子を含む組換えＤＮＡ、該組換えＤＮＡを保有する微生物に関する。ヌクレオシドー５′ーリン酸は、調味料、医薬並びにそれらの原料として有用である。なお、本発明は、Ｘ線結晶構造解析技術により蛋白質の新規立体構造の解明に成功したことに基づくと共に、該立体構造は微生物に限らない発展性を有する。

【０００２】

【従来の技術】ヌクレオシドを生化学的にリン酸化してヌクレオシドー５′ーリン酸を安価かつ効率的に製造する方法として、特定の微生物菌体を、酸性条件下でヌクレオシド並びにポリリン酸（塩）、フェニルリン酸（塩）及びカルバミルリン酸から成る群より選択されるリン酸供与体に作用させることにより、ヌクレオシドー２′ーリン酸、ヌクレオシドー３′ーリン酸異性体の副生を伴うことなくヌクレオシドー５′ーリン酸を効率よく生成する方法が開発されている（特開平７－２３１７９３号）。その後、エシェリヒア・ブラッタエ（Escherichia blattae）及びモルガネラ・モルガニ（Morganella morganii）より酸性ホスファターゼをコードする遺伝子を取得し、遺伝子工学的に該遺伝子をエシェリヒア・コリで大量発現することによりヌクレオシドー５′ーリン酸の生産性が更に向上することが確認された。該酸性ホスファターゼの構造を図２に示す。すなわち、図２は、エシェリヒア・ブラッタエ由来酸性ホスファターゼ（以下、ＥＢ－ＡＰと略記する）のアミノ酸配列を、モルガネラ・モルガニ，サルモネラ・チフィムリウム（Salmonella typhimurium），ザイモモナス・モビリス（Zymomonas mobilis）由来酸性ホスファターゼのアミノ酸配列とアラインメントした図である。それぞれの酸性ホスファターゼの遺伝子の塩基配列と、コードされる酵素のアミノ酸配列を、配列表の配列番号１～８に示す。図２で星印は保存された残基を示す。２次構造の領域をアラインメントの上に棒で示した。四角の線で囲んだ部分は、酸性ホスファターゼファミリーの間で共通しているモチーフを示す。モチーフは、１）KXXXXXXRP配列番号１２１、２）PSGH配列番号１２２、３）SRXXXXXHXXXD配列番号１２３、の３つのドメインから成り立っている。ここで、Xは任意のアミノ酸である。該酸性ホスファターゼ（図２）は、リン酸基転移活性を有するものの、野生型においては、ヌクレオシドー５′ーリン酸をヌクレオシドに分解するホスファターゼ活性が優勢であり、蓄積されたヌクレオシドー５′ーリン酸が分解されてしまう欠点があった。そこで、ランダムに多数の変異型酵素を発生させ、その中からホスファターゼ活性に比してリン酸基転移活性が相対的に向上した変異型酸性ホスファターゼが見出され、該変異型酸性ホスファターゼ遺伝子を大量発現させることによりヌクレオシドー５′ーリン酸の生産性が飛躍的に向上することが示された（特開平９－３７７８５号）。該変異型ホスファターゼは、ヌクレオシドに対する親和性が向上しており、それによりリン酸基転移活性が改善されたと考えられる。上記のエシェリヒア・ブラッタエ由来変異型酸性ホスファターゼ（＝Ｇ７４Ｄ／Ｉ１５３Ｔ変異型酵素）は、モルガネラ・モルガニ由来酸性ホスファターゼ（ＭＭ－ＡＰ）の対応するＧ７２Ｄ／Ｉ１５１Ｔ変異型酵素よりリン酸基転移活性が弱いが、８つのアミノ酸残基を一次構造上対応するＭＭ－ＡＰのアミノ酸に置換した１０残基置換Ｌ６３Ｑ／Ａ６５Ｑ／Ｅ６６Ａ／Ｎ６９Ｄ／Ｓ７１Ａ／Ｓ７２Ａ／Ｇ７４Ｄ／Ｔ１３５Ｋ／Ｅ１３６Ｄ／Ｉ１５３Ｔ変異型酵素（以降、単に１０残基置換変異型ＥＢ－ＡＰと記述）は、Ｇ７２Ｄ／Ｉ１５１Ｔ変異型ＭＭ－ＡＰとほぼ同等のリン酸基転移活性を得ることが示された（特開平１０－２０１４８１号）。

【０００３】

【発明が解決しようとする課題】上述の生産性が向上したエシェリヒア・ブラッタエ由来酸性ホスファターゼ（ＥＢ－ＡＰ）のＧ７４Ｄ／Ｉ１５３Ｔ変異型酵素遺伝子、更には１０残基置換変異型酵素遺伝子をエシェリヒア・コリで大量発現することによりヌクレオシドー５′ーリン酸を生産する方法が確立された（特開平９－３７７８５号、特開平１０－２０１４８１号）が、更に生産性の向上した変異型ＥＢ－ＡＰが望まれる。本発明は、ＥＢ－ＡＰの３次元構造に基づいて、変異型ＥＢ－ＡＰを設計することにより、ヌクレオシドー５′ーリン酸生産性の更なる向上を図ることを課題とする。

【０００４】

【課題を解決するための手段】本発明を概説すれば、下記に列挙するとおりである。

（１）　ヌクレオシドー５′ーリン酸生産酵素において、Lys残基１つ、Arg残基２つ、His残基２つが存在し、これらのＣα間距離が図１に示す範囲内にあり、かつその付近にヌクレオシドが結合するスペースを有する、リン酸基転移活性及び／又はホスファターゼ活性を有する酵素のヌクレオシドー５′ーリン酸生産能が向上した変異型ヌクレオシドー５′ーリン酸生産酵素。

（２）　酵素の由来がエシェリヒア属細菌、モルガネラ属細菌、プロビデンシア属細菌、エンテロバクター属細菌、クレブジエラ属細菌、又はエンテロバクター属細菌に属するものである（１）項記載の変異型ヌクレオシド

－５′－リン酸生産酵素。
（３）　ＥＢ－ＡＰの結晶をＸ線結晶構造解析して得られる原子座標データに示されている構造座標を元にして、イノシン、グアノシン等のヌクレオシドとの結合様式を推定し、アミノ酸残基及び／又は補欠因子等の置換、追加、削除をすることによりヌクレオシド－５′－リン酸生産能が向上した（１）項記載の変異型ヌクレオシド--５′－リン酸生産酵素。
（４）　ＥＢ－ＡＰのアミノ酸配列の以下の位置（ＥＢ－ＡＰのアミノ酸Ser７２、又はSer７２から１０Å以内にある残基）：１６、６７－７６、７８－７９、９６、９９－１００、１０２－１０４、１０６－１０８、１１５、１４０、１４８－１５４、１５７、１７９、１８３の少なくとも一つの位置に変化が生じている変異型ヌクレオシド－５′－リン酸生産酵素。
（５）　リン酸基転移活性及び／又はホスファターゼ活性を有する酵素で、ＥＢ－ＡＰとのアミノ酸配列アラインメントをしたときに、ＥＢ－ＡＰのアミノ酸配列のの以下の位置（ＥＢ－ＡＰのアミノ酸Ser７２、又はSer７２から１０Å以内にある残基）：１６、６７－７６、７８--７９、９６、９９－１００、１０２－１０４、１０６－１０８、１１５、１４０、１４８－１５４、１５７、１７９、１８３の少なくとも一つの位置に変化が生じている変異型ヌクレオシド－５′－リン酸生産酵素。
（６）　リン酸基転移活性及び／又はホスファターゼ活性を有する酵素で、ＥＢ－ＡＰの３次元構造とのアラインメントをトレッディング法により行ったときに、ＥＢ－ＡＰのアミノ酸配列のの以下の位置（ＥＢ－ＡＰのアミノ酸Ser７２、又はSer７２から１０Å以内にある残基）：１６、６７－７６、７８－７９、９６、９９－１００、１０２－１０４、１０６－１０８、１１５、１４０、１４８－１５４、１５７、１７９、１８３の少なくとも一つの位置に変化が生じている変異型ヌクレオシド－５′－リン酸生産酵素。
【０００５】（７）　酵素の由来がエンテロバクター・アェロゲネス由来であり、そのアミノ酸配列において１４番目のロイシン残基、６１番目のロイシン残基、６３番目のアラニン残基、６４番目のグルタミン酸残基、６７番目のアスパラギン残基、６９番目のセリン残基、７０番目のアラニン残基、７１番目のグリシン残基、７２番目のグリシン残基、１０１番目のイソロイシン残基、１０２番目のグルタミン酸残基、１３３番目のスレオニン残基、１３４番目のグルタミン酸残基、１３８番目のロイシン残基、１４９番目のスレオニン残基、１５１番目のイソロイシン残基のうち少なくとも一つのアミノ酸残基が他のアミノ酸残基に置換されたものである変異型ヌクレオシド－５′－リン酸生産酵素。
（８）　リン酸基転移活性及び／又はホスファターゼ活性を有する酵素、あるいはそれとモリブデン酸との複合体の結晶をＸ線結晶構造解析して得られる立体構造から確定された、該酵素の活性部位に位置するアミノ酸残基（Lys１つ、Arg２つ、His２つにより構成される）、及び／又はそれから１０Å以内にあるアミノ酸残基を、置換、追加、削除をすることにより、ヌクレオシド－５′－リン酸生産能が向上した変異型酵素を製造することを特徴とする変異型ヌクレオシド－５′－リン酸生産酵素の製造方法。
（９）　エシェリヒア・ブラッタエ由来酸性ホスファターゼの構造座標を使用して、ホスファターゼ又はリン酸基転移酵素の阻害剤を製造する方法。
【０００６】（１０）　リン酸基転移活性及び／又はホスファターゼ活性を有する酵素、あるいはそれとモリブデン酸との複合体のいずれかの結晶。
（１１）　六方晶系の空間群$P6_322$を有する、ＥＢ－ＡＰの結晶。
（１２）　斜方晶系の空間群$P2_12_12_1$を有する、ＥＢ－ＡＰ　Ｇ７４Ｄ／Ｉ１５３Ｔ変異型酵素の結晶。
（１３）　三方晶系の空間群$P3_121$を有する、ＥＢ－ＡＰとモリブデン酸との複合体（反応中間体アナログ）の結晶。
【０００７】（１４）　（１）～（７）項のいずれか１項に記載の酵素をコードする遺伝子。
（１５）　（１４）項に記載の遺伝子を含む組換えＤＮＡ。
（１６）　（１５）項に記載の組換えＤＮＡを保有する微生物。
【０００８】（１７）　（１）～（７）項のいずれか１項に記載の酵素、又はそれを含有する微生物、あるいは（１６）項に記載の微生物を、ヌクレオシド並びにリン酸供与体に作用させてヌクレオシド－５′－リン酸を生成させ、これを採取することを特徴とするヌクレオシド－５′－リン酸の製造方法。
【０００９】本発明は、ＥＢ－ＡＰの３次元構造を基に、ヌクレオシドとの結合様式モデルを構築し、それに基づき設計した変異型ＥＢ－ＡＰを利用したヌクレオシド－５′－リン酸の生産方法を提供する。
【００１０】
【発明の実施の形態】以下、本発明を具体的に説明する。
（１）　Ｘ線結晶構造解析により蛋白質の３次元構造を決定するには、蛋白質を結晶化する必要がある（実施例１－３に詳細を示した）。蛋白質を結晶化するためには、ｐＨ、バッファーの種類、バッファーの濃度、沈殿剤の種類、沈殿剤の濃度、金属等の添加剤の濃度、蛋白質の濃度、蛋白質の純度、等、数多くのパラメーターを試行錯誤により決定しなくてはならない。したがって、結晶を得るまでに数ヶ月～数年の時間がかかるのが通常であり、多大な労力に反して結晶が得られないケースも多々ある。結晶化は、３次元構造決定のためには欠かせないが、それ以外にも、蛋白質の高純度の精製法、高密

度でプロテアーゼ抵抗性の強い安定な保存法、更には酵素の固定化利用に先立つプロセスとして産業上の有用性もある。

（２）　作製した結晶にＸ線を照射して回折データを収集する。蛋白質結晶はＸ線照射によりダメージを受け回折能が劣化するケースが多々ある。その場合、結晶を急激に－１７３℃程度に冷却し、その状態で回折データを収集する低温測定が最近普及しつつある。なお、冷却に際しては、結晶が崩壊せず系全体がガラス状になるよう溶媒組成を工夫する必要がある。

（３）　結晶構造解析を行うには、回折データに加えて、位相情報が必要になる。ＥＢ－ＡＰは、類縁の蛋白質の立体構造が未知であるため、重原子同型置換法により位相問題が解決されなくてはならない。重原子同型置換法は、水銀や白金等原子番号が大きな金属原子を結晶に導入し、金属原子の大きなＸ線散乱能のＸ線回折データへの寄与を利用して位相情報を得る方法である。野生型ＥＢ－ＡＰの立体構造が決定されれば、変異型酵素及び反応中間体アナログ等類縁体の結晶構造は、それを用いた分子置換法により決定できる。分子置換法は、結晶構造を決定したい蛋白質に類縁の蛋白質の立体構造が既知の場合、その立体構造を利用して構造決定を行う手法である。例えば、ある蛋白質の野生型の立体構造が分かっていれば、その変異型蛋白質や化学修飾された蛋白質の結晶構造決定には、分子置換法が適用できる。Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰについては、２ヶ所のアミノ酸置換によるヌクレオシド親和性向上の分子機構解明のために結晶構造を決定する。反応中間体アナログについては、ヌクレオシドとの結合様式モデルを構築するために結晶構造を決定する。ヌクレオシドは、供与されるリン酸基が共有結合した状態のＥＢ－ＡＰ、すなわち反応中間体に結合した後、ヌクレオシド－５′－リン酸に変換される。ＥＢ－ＡＰの反応中間体は不安定なので、その構造を捉えることができないが、リン酸の代りにモリブデン酸が共有結合した反応中間体アナログであれば加水分解されることがないので構造決定可能と考えた。実施例４、６、７に詳細を示した。

【００１１】（４）　コンピューターグラフィックス（ＣＧ）上で、反応中間体アナログの３次元構造におけるモリブデン酸結合位置を基に、その付近の窪みにヌクレオシドをフィットさせ、結合様式モデルを構築する（図３）。モデルの構築には、例えばＭＳＩ社（アメリカ）のQUANTA, INSIGHT IIの様なプログラムを利用する。なお、図３は、上記の結合様式モデルの結晶構造を示す写真である。実施例５、８に詳細を示した。

（５）　結合モデルを良く観察し、ヌクレオシドとの親和性を増大させる変異を設計する。親和性を向上させるには、疎水相互作用・静電相互作用・水素結合・π－π相互作用（芳香環の環電流が発生する磁場同士の相互作用）・ＣＨ／π相互作用（芳香環の環電流とメチル基の電子が発生する磁場の相互作用）を増強する手段が考えられる。Ser７２はヌクレオシドの塩基と最も強く相互作用すると予測されるため、Phe、Tyr、Trpへの置換は疎水性相互作用及びπ－π相互作用を、Va１、Ile、Ｌｅｕへの置換は疎水性相互作用及びCH／π相互作用をGlu、Aspへの置換は静電相互作用及び水素結合を増強するものと思われる。また、他のアミノ酸への置換、特により長鎖の側鎖を有するアミノ酸への置換によっても疎水性相互作用等が増強される可能性がある。Ｌｅｕ１６、Ser７１、Ser７３、Glu１０４をPhe、Tyr、Trpに置換することによっても置換されたアミノ酸残基の芳香環とヌクレオシドの塩基との間にπ－π相互作用が形成されることが期待される。またIle１０３やThr１５３をより長鎖の親水性残基に置換することによりヌクレオシドのリボースとの水素結合の形成が期待される。更に、ヌクレオシド結合部位の近くに位置し、蛋白質内部に埋もれているThr１５１をSer、Ala、Glyといった小さな側鎖を持ったアミノ酸残基に置換すれば、蛋白質内部に空隙が生じるために、ヌクレオシド結合部位の柔軟性が増し、ヌクレオシドとの結合により適したコンフォメーションを取れるのではないかと期待される。なお、Ｌｅｕ１４０は、Ser７２から１０Å以上離れているが、反応中間体アナログの立体構造において、リン酸結合部位の直近に位置する。したがって、この残基を置換すれば、反応中間体におけるリン酸結合部位周辺の構造が変化し、ひいては、ヌクレオシド結合部位の構造と揺らぎにも影響が及ぶものと考えられた。この残基を、よりかさ高いPhe、正電荷を有するLys、負電荷を有するGlu、に置換すれば、ヌクレオシドとの親和性が変化するのではないかと期待される。上記の変異は、当初はＧ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰに対して導入する。しかし、導入の対象とする変異型酵素は、Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰに限られるものではない。例えば、１０残基置換変異型ＥＢ－ＡＰに変異を導入することも可能である。変異は、Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰに対して導入することにする。この場合、作製される変異型酵素は、３残基置換変異型酵素となる。実施例９に詳細を示した。

（６）　ＰＣＲ法により、変異型ＥＢ－ＡＰをコードする遺伝子を含むプラスミドを作製する。プラスミドをエシェリヒア・コリ（Escherichia coli）ＪＭ１０９に導入し、変異型ＥＢ－ＡＰを生産させる。変異型ＥＢ－ＡＰのイノシンに対する親和性の指標になるＫｍ値、及びイノシンを５′－イノシン酸に変換するリン酸基転移活性を測定し、変異型ＥＢ－ＡＰの性能を評価する。ヌクレオシド－５′－リン酸の生産量は、多分にＫｍ値に依存すると考えられる。ピロリン酸がＥＢ－ＡＰと反応し、リン酸イオンが離脱し、リン酸基がＥＢ－ＡＰと共有結合した形の反応中間体が形成された後、水分子がこれを攻撃すれば、リン酸基が外れてしまう（ホスファタ

ーゼ反応）。ピロリン酸は、ヌクレオシドー５′ーリン酸を生成せずに無駄に消費されたことになる。一方、ヌクレオシドが反応中間体を攻撃すれば、リン酸基はヌクレオシドとホスホモノエステル結合を形成し、生成したヌクレオシドー５′ーリン酸がＥＢ－ＡＰから離脱する（リン酸基転移反応）。ピロリン酸はヌクレオシドー５′ーリン酸生成に活用されたことになる。つまり、反応中間体を水とヌクレオシドが取り合い水が勝てばホスファターゼ活性が、ヌクレオシドが勝てばリン酸基転移活性が、発揮されることになる。ヌクレオシドのＥＢ－ＡＰに対する親和性が上昇すれば、すなわち、Ｋｍ値が低下すれば、リン酸基転移反応が行われる可能性が高くなる。また、リン酸結合部位付近の疎水性を高め、水が近づきにくくなれば、ホスファターゼ活性が弱まり、リン酸基転移活性が相対的に強まることになる。実施例１０に詳細を示した。

（７）　Ｋｍ値が低下し、かつリン酸基転移活性が上昇した変異型ＥＢ－ＡＰ遺伝子を含むプラスミドを導入したエシェリヒア・コリＪＭ１０９を用いて、イノシンから５′ーイノシン酸を生産する実験を行う。３０℃で４５時間反応を行い５′ーイノシン酸生産量の経時変化をモニターする。実施例１２に詳細を示した。

（８）　Ｋｍを低下させる変異箇所が見出されたら、複数の残基を組合せることによって更にヌクレオシドに対する親和性が向上し、生産性の高くなった変異型酵素を作製することができる。累加的に部位特異的変異を繰り返すことで複数の変異部位を導入することができる。また部位特異的変異の導入の際に、変異を導入するアミノ酸残基をコードする塩基の部分がミックス塩基となったプライマーを用いると特定のアミノ酸残基がすべてのアミノ酸に置換された変異型遺伝子のライブラリーを作成することができる。複数の部位にミックス塩基のプライマーを用いて変異を導入すると非常に多種類の変異型酵素をコードする変異型遺伝子のライブラリーを作成することができる。このようにして構築した遺伝子のライブラリーをエシェリヒア・コリに導入し、発現させたライブラリーから高活性のアミノ酸置換が組合された変異体を選び出す方法も有効である。

【００１２】ＥＢ－ＡＰ以外でも、ＥＢ－ＡＰと同様の活性部位及びヌクレオシドが結合しうるスペースを持つ酵素であれば、ヌクレオシドー５′ーリン酸の生産に利用できるポテンシャルがある。活性部位は、活性に必須なアミノ酸残基を有し、かつ、それらが適切な空間的位置関係で配置されていなくてはならない。ＥＢ－ＡＰにおいては、Ｌｙｓ１１５，Ａｒｇ１２２，Ｈｉｓ１５０，Ａｒｇ１８３，Ｈｉｓ１８９が活性に必須であり、これら５残基のＣα間距離によって空間的位置関係を規定することが可能である。本発明においては、野生型、Ｇ７４Ｄ／Ｉ１５３Ｔ変異型、反応中間体アナログの３つのＥＢ－ＡＰ結晶構造を決定したので、それぞれの構造における活性残基のＣα原子間距離を測り、表１を作成した。表１の各々の距離分布が約１Åの幅を持つことから、最短距離より１Å短い距離（表１、下限）以上、最長距離より１Å長い距離（表１、上限）以下であれば、求められる活性部位を形成できるものと考えた。図１に、５つの残基の位置関係を上限、下限のCα間距離と共に示した。なお、ＥＢ－ＡＰの類縁酵素であるＭＭ－ＡＰにおいて、活性残基間のすべての原子間距離が、ＥＢ－ＡＰの立体構造から規定した範囲におさまっていることが確認されたことを実施例１５に示した。本実施例では、野生型ではなく、Ｇ７２Ｄ／Ｉ１５１Ｔ変異型の例を示したが、同じ酵素の野生型と変異型で、活性部位の立体構造に大きな違いはないと考えられる。この推測は、ＥＢ－ＡＰの野生型とＧ７４Ｄ／Ｉ１５３Ｔ変異型の活性部位の構造が基本的に同じであることにより支持される（表１参照）。ヌクレオシドをリン酸化して、ヌクレオシドー５′ーリン酸に変換するには、上記の５残基から成る活性部位だけでは不充分で、ヌクレオシドが適切な位置に結合できなくてはならない。ＥＢ－ＡＰの場合は、リン酸基の結合部位付近に、ヌクレオシドが結合するのに適した溝状のスペースが分子表面に存在する〔（図３）：添付した原子座標を示す図１０～図４５を用いて、コンピュータグラフィックス（ＣＧ）上で表示できる。〕この溝は、Ｌｅｕ１６、Ｓｅｒ７２、Ｇｌｕ１０４、Ｈｉｓ１８９の４残基によって囲まれるスペースとして規定される。活性部位を有していても、ヌクレオシドが結合する適切なスペースがない酵素は、ヌクレオシドー５′ーリン酸生産酵素としては不適である。

【００１３】

【表１】

		野生型	G74D/I153T 変異型	反応中間体アナログ	下限	上限
Lys115	Arg122	11.6Å	11.6Å	11.4Å	10.4Å	12.6Å
	His150	12.4Å	12.3Å	12.8Å	11.3Å	13.8Å
	Arg183	16.4Å	16.3Å	15.5Å	14.5Å	17.4Å
	His189	12.6Å	12.1Å	11.7Å	10.7Å	13.6Å
Arg122	His150	13.2Å	13.6Å	14.2Å	12.2Å	15.2Å
	Arg183	10.4Å	10.5Å	10.8Å	9.4Å	11.8Å
	His189	5.6Å	5.5Å	5.7Å	4.5Å	6.7Å
His150	Arg183	8.4Å	8.8Å	7.7Å	6.7Å	9.8Å
	His189	9.8Å	10.0Å	10.0Å	8.8Å	11.0Å
Arg183	His189	5.5Å	5.8Å	5.7Å	4.5Å	6.8Å

【００１４】また、本発明は、Ser７２の他のアミノ酸への置換、好ましくはPhe、Tyr、Trp、Val、Leu、Glu、Asp、Gln、Met、Thr、Arg、Lysのいずれか一つのアミノ酸への置換、を施した変異型ＥＢ－ＡＰを提供する。更に、Ser７２から１０Å以内にある残基（残基番号：１６、７０－７１，７３－７６、１００、１０２－１０４、１０６－１０８、１１５、１４８－１５４、１８３）はヌクレオシドと相互作用する可能性が非常に高く、これらアミノ酸残基の他のアミノ酸への置換を施した変異型ＥＢ－ＡＰを提供する。ここで置換とは、人為的にアミノ酸を置換した場合のみならず、自然界において置換が生じたＥＢ－ＡＰと同じ酵素ファミリーに属する他の酸性ホスファターゼを選抜することも包含する。ただし、本発明は、上記のアミノ酸残基以外の変異点を含む変異型ＥＢ－ＡＰも提供できる。

【００１５】また、ＥＢ－ＡＰと同じ酵素ファミリーに属する他の酸性ホスファターゼにおいても、相同のアミノ酸変異を施せば、ヌクレオシド－５′－リン酸の製造に利用することが可能である。ただし、ＥＢ－ＡＰのアミノ酸残基が、他の酸性ホスファターゼにおいて同じ番号のアミノ酸残基に対応するとは限らない。例えば、ＥＢ－ＡＰのSer７２は、ＭＭ－ＡＰにおいてはAla７０に対応する。２つの異なった蛋白質のアミノ酸残基の対応付けは、両者のアミノ酸配列の相同性が２０％程度以上であればアミノ酸配列同士のアラインメント（Sequence Alignment）、２０％程度以下であれば３次元構造とアミノ酸配列のアラインメント（Threading）により判明する。前者はBLAST等、後者はINSIGHT II等のプログラムにより実行が可能である。BLASTを用いた、ＥＢ－ＡＰとエンテロバクター・アエロゲネス（Enterobacter aerogenes）由来酸性ホファターゼ（ＥＡ－ＡＰ）のアミノ酸配列アラインメントを実施例１４に示した。BLASTは、FTPを使って、ncbi.nlm.nih.govより／blast／executableに存在するファイルのうち、使用するコンピューターに則したファイルを入手すればよい。操作法については、http：／／genome. nhgri. nih. gov／blastall／blast#installに詳細が記述されている。

【００１６】ヌクレオシド－５′－リン酸生産能向上は、ヌクレオシドとの親和性向上により達成されるケースが多いが、それ以外にも、至適ｐＨのシフト、熱安定性の向上、等によっても達成されうる。至適ｐＨのシフトは、活性残基のｐＫを変えることで達成できる〔プロティン　エンジニアリング(Protein Engng.)、第1巻、第383～388頁(1998)〕。熱安定性の向上は、プロリン残基の導入、左巻きヘリックス構造を取る残基のグリシン残基への置換〔プロティン　エンジニアリング、第６巻、第８５～９１頁(1993)〕、蛋白質内部の空隙を埋めること〔バイオケミストリー(Biochemistry)、第32巻、第6171～6178頁(1993)〕等により達成可能である。以上詳細に説明したように、該立体構造はヌクレオシドの親和性が向上し、ヌクレオシド－５′－リン酸生産能の向上した変異体を作製するために有効であるが、該立体構造は酵素のヌクレオシドに対する親和性のみならず、リン酸供与体との親和性を変化させるのにも有効である。特開平９－３７７８５号公報に記載されているように該酵素はポリリン酸（塩）、フェニルリン酸（塩）、アセチルリン酸（塩）及びカルバミルリン酸（塩）等各種リン酸エステル化合物をリン酸供与体として利用することが可能であるが、ヌクレオシドとの親和性を増大させる変異を設計したのと同様の方法にて、リン酸エステル化合物との親和性を増大させる変異を設計することで、リン酸供与体の基質特異性を広げたり、リン酸の利用率を向上させることが可能である。

【００１７】

【実施例】以下、本発明を実施例により更に具体的に説明するが、本発明はこれらの実施例に限定されない。

【００１８】実施例１　野生型ＥＢ-ＡＰの結晶化

ハンギングドロップ法での蒸気拡散を利用して結晶化を行った。野生型ＥＢ-ＡＰ（濃度１０ｍｇ／ｍｌ）を含むリン酸ナトリウムの２０ｍＭ緩衝液(ｐＨ８．０)と、

４５（ｗ／ｖ％）のポリエチレングリコール４００を含むトリス塩酸の１００ｍＭ緩衝液を同量ずつ（各々７～１０μｌ）、シリコナイゼイションしたカバーグラス上に滴下混合し、４５（ｗ／ｖ％）のポリエチレングリコール４００を含むトリス塩酸の１００ｍＭ緩衝液５００μｌを満たしたウエル（well）の上に混合液滴が釣り下がるようにかぶせ、２０℃にて静置した。２、３日後に結晶が析出し、１週間から２週間後には測定可能な大きさ（０．３×０．３×１．２mm程度）の六角柱状の結晶に成長した。Ｘ線データ測定の際には、５０（ｗ／ｖ％）のポリエチレングリコール４００を含むトリス塩酸の１００ｍＭ緩衝液（ｐＨ８．０）に結晶を移した。この結晶は、取扱い上、次の点に留意する必要があった。１）液滴（ドロップレット）から、結晶を取り出す際に容器や用具に接触することで結晶が非常に崩れやすいため、シッティングドロップ法の結晶化形態は用いることが出来ず（結晶は成長するが）、ここで述べたハンギングドロップ法を用いた。２）常温測定では、測定中に結晶が劣化し、徐々に分解能が下がるため、低温条件下での測定が必要であった。結晶をステージにマウントするまでの時間を極力短くし、空気にさらさないように工夫した。（株）リガクのＸ線回折装置R-AXIS IIcを用いて、Ｘ線回折データを収集し、結晶学的パラメーターを決定した。空間群は$P6_322$、格子定数は、ａ＝ｂ＝１２４．４Å、ｃ＝９７．７Åとなった。非対称単位に分子量２５０００のサブユニットを一つ含むと仮定すると、結晶の水分含有率は７２％となる。

【００１９】実施例２　Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ-ＡＰの結晶化

ハンギングドロップ法での蒸気拡散を利用して結晶化を行った。Ｇ７４Ｄ／Ｉ１５３Ｔ変異型酵素（濃度２０ｍｇ／ｍｌ）を含むトリス塩酸の２０ｍＭ緩衝液（ｐＨ８．０）と、３８（ｗ／ｖ％）のポリエチレングリコール４００を含むトリス塩酸の２０ｍＭ緩衝液を同量ずつ（各々５μｌ）、シリコナイゼイションしたカバーグラス上に滴下混合し、３８（ｗ／ｖ％）のポリエチレングリコール４００を含むトリス塩酸の２０ｍＭ緩衝液５００μｌを満たしたウエルの上に混合液滴が釣り下がるようにかぶせ、２０℃にて静置した。２、３日後に結晶が析出し、１週間後には測定可能な大きさ（０．７×０．４×０．２mm程度）の板状結晶に成長した。Ｘ線データ測定の際には、５０（ｗ／ｖ％）のポリエチレングリコール４００を含むトリス塩酸の１００ｍＭ緩衝液（ｐＨ８．０）に結晶を移した。（株）リガクのＸ線回折装置R-AXIS IIcを用いて、Ｘ線回折データを収集し、結晶学的パラメーターを決定した。空間群は$P2_12_12_1$、格子定数はａ＝１３８．０Å、ｂ＝１６８．３Å、ｃ＝５８．２Åとなった。非対称単位に分子量１５００００の６量体分子を一つ含むと仮定すると、結晶の水分含有率は６４％となる。

【００２０】実施例３　野生型ＥＢ-ＡＰとモリブデン酸との複合体（反応中間体アナログ）の結晶化

シッティングドロップ法での蒸気拡散を利用した共結晶化法を用いて結晶化を行った。野生型ＥＢ-ＡＰ（濃度１０ｍｇ／ｍｌ）を含むリン酸ナトリウムの２０ｍＭ緩衝液（ｐＨ８．０）と、４０（ｗ／ｖ％）のポリエチレングリコール４００、及び１ｍＭのモリブデン酸ナトリウムを含むトリス塩酸の１００ｍＭ緩衝液を同量ずつ（各々１５μｌ）を、４０（ｗ／ｖ％）のポリエチレングリコール４００を含むトリス塩酸の１００ｍＭ緩衝液（ｐＨ８．０）５００μｌを満たしたウエルに設置したブリッジの窪みに滴下混合し、２０℃にて静置した。２～３日後に結晶が析出し、１週間から２週間後には測定可能な大きさ（０．３×０．３×０．３㎜程度）の菱餅状の結晶に成長した。Ｘ線データ測定の際には、５０（ｗ／ｖ％）のポリエチレングリコール４００を含むトリス塩酸の１００ｍＭ緩衝液（ｐＨ８．０）に結晶を移した。(株)リガクのＸ線回折装置R-AXIS IIcを用いて、Ｘ線回折データを収集し、結晶学的パラメーターを決定した。空間群は$P3_121$、格子定数はａ＝ｂ＝８６．６Å、ｃ＝２０５．３Åとなった。非対称単位に分子量２５０００のサブユニットを３つ含むと仮定すると、結晶の水分含有率は５８％となる。

【００２１】実施例４　野生型ＥＢ-ＡＰの結晶構造解析

最高１．９Å分解能データまでのＸ線回折データを測定した。結晶は、常温においては、Ｘ線の照射によるダメージが激しかったので、－１７３℃に急速冷却して測定を行った。重金属塩類の溶液中に結晶を浸すことにより、重原子誘導体のスクリーニングを行った。重原子誘導体結晶の回折データはリガクR-AXIS IIcを用いて得た。ネイティブデータとの差フーリエ図より、K_2PtCl_4が良好な重原子同型結晶を与えることを見出した。プログラムＲＳＰＳを用いることにより、K_2PtCl_4の唯一のプラチナ結合部位の座標を求めた。この座標をプログラムMLPHAREにより精密化し、それから計算される位相を求めた。この位相を用い、２つ目の重原子誘導体$KHgI_4$－ＫＩの５カ所の水銀結合部位を求めた。K_2PtCl_4、$KHgI_4$－ＫＩ両方の重原子パラメーターをMLPHAREを用いて同時に精密化した後、プログラムＤＭを用いて、溶媒平滑化とヒストグラムマッチングを行い、位相を改良した。ちなみに、K_2PtCl_4については異常分散データも使用した。この良好な位相を用いて計算した電子密度マップは非常に鮮明で、ほとんどのアミノ酸残基をきれいにフィットすることができた。最初のモデルは、２．８Å分解能で作成した電子密度マップ上でプログラムQUANTAを用いて構築し、プログラムＸ-ＰＬＯＲを用いて構造精密化を行った。Ｎ末の６残基、１３５－１３６番目の残基、Ｃ末の１残基は電子密度が観測されず、構造を一義的に決定できなか

った。１．９Å分解能で精密化された最終モデル（図４～図６）は、全２３１残基中２２２残基、２３６個の水分子、１分子の硫酸イオンを含む。硫酸イオンは、精製過程で用いた硫酸アンモニウムに由来しており、活性中心のリン酸結合部位に一致するものと考えられる。８～１．９Å分解能の反射を用いた結晶学的信頼度因子（R因子）は２１．５%となった。平均の温度因子は、蛋白質原子について２６Å^2、水分子について４５Å^{2}となった。プログラムPROCHECKを用いてラマチャンドランプロットを作成したところ、グリシン以外の残基の９３%が最も好ましい領域に、７%が次に好ましい領域に位置することが示された。非対称単位にはサブユニット１個が含まれ、結晶学的対称性によって６量体が形成される。原子座標は図１０～図４５に示した。

【００２２】なお、図４は、ＥＢ－ＡＰの６量体分子の結晶構造を示すＣＧ写真である。α炭素原子の流れをリボンモデルで表示した。また、活性中心をマークする硫酸イオンをボールモデルで表示した。図５は、ＥＢ－ＡＰのサブユニットの結晶構造を示すＣＧ写真である。α炭素原子の流れをリボンモデルで表示した。また、活性中心をマークする硫酸イオンをボールモデルで表示した。図６は、ＥＢ－ＡＰの活性部位構造を示す図である。中央に硫酸イオンを示した。また、水素結合を点線で示した。図１０は、ＥＢ－ＡＰの構造の結晶学データ（１）を示す図である。図１１は、ＥＢ－ＡＰの構造の結晶学データ（２）を示す図である。図１２は、ＥＢ－ＡＰの構造の結晶学データ（３）を示す図である。図１３は、ＥＢ－ＡＰの構造の結晶学データ（４）を示す図である。図１４は、ＥＢ－ＡＰの構造の結晶学データ（５）を示す図である。図１５は、ＥＢ－ＡＰの構造の結晶学データ（６）を示す図である。図１６は、ＥＢ－ＡＰの構造の結晶学データ（７）を示す図である。図１７は、ＥＢ－ＡＰの構造の結晶学データ（８）を示す図である。図１８は、ＥＢ－ＡＰの構造の結晶学データ（９）を示す図である。図１９は、ＥＢ－ＡＰの構造の結晶学データ（１０）を示す図である。図２０は、ＥＢ－ＡＰの構造の結晶学データ（１１）を示す図である。図２１は、ＥＢ－ＡＰの構造の結晶学データ（１２）を示す図である。図２２は、ＥＢ－ＡＰの構造の結晶学データ（１３）を示す図である。図２３は、ＥＢ－ＡＰの構造の結晶学データ（１４）を示す図である。図２４は、ＥＢ－ＡＰの構造の結晶学データ（１５）を示す図である。図２５は、ＥＢ－ＡＰの構造の結晶学データ（１６）を示す図である。図２６は、ＥＢ－ＡＰの構造の結晶学データ（１７）を示す図である。図２７は、ＥＢ－ＡＰの構造の結晶学データ（１８）を示す図である。図２８は、ＥＢ－ＡＰの構造の結晶学データ（１９）を示す図である。図２９は、ＥＢ－ＡＰの構造の結晶学データ（２０）を示す図である。図３０は、ＥＢ－ＡＰの構造の結晶学データ（２１）を示す図である。図３１は、ＥＢ－ＡＰの構造の結晶学データ（２２）を示す図である。図３２は、ＥＢ－ＡＰの構造の結晶学データ（２３）を示す図である。図３３は、ＥＢ－ＡＰの構造の結晶学データ（２４）を示す図である。図３４は、ＥＢ－ＡＰの構造の結晶学データ（２５）を示す図である。図３５は、ＥＢ－ＡＰの構造の結晶学データ（２６）を示す図である。図３６は、ＥＢ－ＡＰの構造の結晶学データ（２７）を示す図である。図３７は、ＥＢ－ＡＰの構造の結晶学データ（２８）を示す図である。図３８は、ＥＢ－ＡＰの構造の結晶学データ（２９）を示す図である。図３９は、ＥＢ－ＡＰの構造の結晶学データ（３０）を示す図である。図４０は、ＥＢ－ＡＰの構造の結晶学データ（３１）を示す図である。図４１は、ＥＢ－ＡＰの構造の結晶学データ（３２）を示す図である。図４２は、ＥＢ－ＡＰの構造の結晶学データ（３３）を示す図である。図４３は、ＥＢ－ＡＰの構造の結晶学データ（３４）を示す図である。図４４は、ＥＢ－ＡＰの構造の結晶学データ（３５）を示す図である。図４５は、ＥＢ－ＡＰの構造の結晶学データ（３６）を示す図である。

【００２３】実施例５　野生型ＥＢ－ＡＰと5′-イノシン酸の結合様式モデルの推測

イノシンのＥＢ－ＡＰに対するＫｍ値は１００ｍＭを超えることから、結合様式をＸ線結晶構造解析で決定できるほど親和性が高くない。実際に、グルコース－６－サルフェートやアデノシン－チオモノホスフェートといったＥＢ－ＡＰの阻害剤となる化合物を野生型ＥＢ－ＡＰの結晶にソーキングした後、Ｘ線回折データを収集し、電子密度マップを作成したが、これら化合物に対応する電子密度は観測されなかった。そこで、コンピュータグラフィックスを用いて、5′－イノシン酸とＥＢ－ＡＰの結合様式の推測（いわゆる、ドッキングスタディ）を行うこととした。プログラムはQUANTAを用いた。結晶構造中、活性部位中央に硫酸イオンが見出されたので、ここに、5′－イノシン酸のリン酸基を重ね合せた。更に、Ｇ７４Ｄ及びＩ１５３Ｔの変異が5′－イノシン酸のＥＢ－ＡＰに対するＫｍ値を低下させることが知られていたので、5′－イノシン酸はＧ７４及びＩ１５３から遠くないところに結合すると判断し、5′－イノシン酸の位置を決めた。その際、5′－イノシン酸を構成する原子とＥＢ－ＡＰを構成する原子がお互いにぶつからないようにした。こうして構築したモデルにおいて、Ｉ１５３がＴになると、置換されたスレオニンの側鎖のγ酸素原子とイノシンのリボースの2′水酸基が水素結合を形成する。また、プログラムGRASPを用いてＥＢ－ＡＰの静電ポテンシャル表示をしたところ、正電荷を帯びるイノシン塩基は、ＥＢ－ＡＰ分子表面で負電荷を帯びている領域と相互作用しており、モデルがもっともらしいことが示唆された。

【００２４】実施例６　Ｇ７４Ｄ／Ｉ１５３Ｔ変異型Ｅ

ＥＢ－ＡＰの結晶構造解析
Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰは、ホスファターゼ活性に対するリン酸基転移活性の比率が高まっており、それに伴い、ヌクレオシドー５′--リン酸の生産能力も向上している。これは、ヌクレオシドとのＫｍ値が低下したこと、すなわち、ヌクレオシドとの親和性が向上したことが原因として考えられている。この変異型ＥＢ－ＡＰの結晶構造を決定し、野生型ＥＢ－ＡＰの結晶構造と比較すれば、ヌクレオシドとの親和性向上の分子機構が解明されることを期待した。常温で、最高２．４Å分解能データまでのＸ線回折データを測定した。単位格子の体積、空間群、酵素の分子量から見積もって、非対称単位には６量体の分子１つが含まれることが予想された。そこで、野生型ＥＢ－ＡＰの６量体構造を探索モデルとして、プログラムamoreを用いて分子置換法により解析を行った。rotation searchにおいては１０～３Å分解能のデータを、translation searchにおいては１０～４Å分解能のデータを用いた。両サーチともに、正解がトップピークとして現れた。分子を剛体として精密化を行ったところ、Ｒ因子は３７．３%に低下した。この後、QUANTAを用いたグラフィックス上での構造修正とX-PLORを用いた構造精密化を繰り返し行い、１０～２．４Å分解能において、Ｒ因子１９．９%のモデルを得た。実施例５と同様の方法で、5′-イノシン酸とＧ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰの結合モデルを作成したところ、置換されたThr１５３の側鎖のγ酸素原子は、イノシンのリボースの水酸基と水素結合を形成することが予想された。また、もう一つ置換が施されたAsp７４を含むループの揺らぎが、野生型に比べＧ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰの方が大きくなっていることが、温度因子を比較することにより分かった。このループはイノシンの塩基と相互作用することが予想されるが、揺らぎが大きくなったことにより塩基との結合がしやすくなった可能性が示唆される。

【００２５】実施例７　野生型ＥＢ－ＡＰとモリブデン酸との複合体（反応中間体アナログ）の結晶構造解析
ＥＢ－ＡＰの酵素反応において、まず初めに、リン酸モノエステル結合が切断され、リン酸基は活性残基のHis１８９と共有結合を形成する。この状態の酵素分子を反応中間体と呼ぶ。反応中間体は速やかに、水あるいはアルコールによるアタックを受け、その結果、リン酸イオンが離脱する。水がアタックすればホスファターゼ活性が発揮されることとなり、また、アルコールがアタックすればリン酸基転移活性が発揮されることになる。いずれにしても、反応中間体は不安定であり、その構造をＸ線結晶構造解析により決定することは不可能である。しかし、リン酸の代わりにモリブデン酸がHis１８９に共有結合したもの（反応中間体アナログ）は、水によるアタックを受けないので安定に存在する。リン酸基転移反応においては、反応中間体にリン酸受容体が結合し、リン酸モノエステル結合が形成される。したがって、ヌクレオシドとの結合様式を推定する目的においては、遊離型構造よりも、反応中間体構造を用いる方が適切である。反応中間体とヌクレオシドとのドッキングスタディを行う目的で、反応中間体アナログの結晶構造解析を行った。常温にて、最高２．４Å分解能データまでのＸ線回折データを測定した。単位格子の体積、空間群、酵素の分子量から見積もって、非対称単位には６量体の半分、すなわち、サブユニット３つが含まれることが予想された。そこで、３回軸によってお互いが関係づけられる３量体構造を作成し、分子置換法の探索モデルとした。rotation searchにおいては１０～３Å分解能のデータを、translationsearchにおいては１０～４Å分解能のデータを用いた。両サーチともに、正解がトップピークとして現れた。分子を剛体として精密化を行ったところ、Ｒ因子は４２．４%に低下した。この後、QUANTAを用いたグラフィックス上での構造修正とＸ－ＰＬＯＲを用いた構造精密化を繰り返し行い、８～２．４Å分解能において、Ｒ因子２２．３%のモデルを得た。非対称単位には６量体の分子半分、つまりサブユニット３つが含まれる。

【００２６】実施例８　ＥＢ－ＡＰ反応中間体とイノシンの結合様式モデルの推測
コンピュータグラフィックス上、QUANTAを用いて、結合様式モデルを構築した（図３）。モリブデン酸はそのままリン酸に置き換えた。イノシンは、野生型ＥＢ－ＡＰと5′－イノシン酸の結合様式モデルにおける5′-イノシン酸のヌクレオシド部分近くに置いた。ただし、当然のことだが、イノシンはリン酸モノエステル結合を持たないので、5′－イノシン酸をドッキングさせるよりも自由度が高い。したがって、イノシンがＥＢ－ＡＰの分子表面に、より好ましい状態で結合するように、イノシンの位置の微調整を行い、結合様式モデルとした。以降の変異型酵素のデザインには、このモデルを用いることとした。

【００２７】実施例９　ヌクレオシドとの親和性向上を目指した変異型ＥＢ－ＡＰのデザイン
実施例８で構築したモデルによると、Ser７２の側鎖は、イノシンの塩基と相互作用する可能性が示唆された。この残基をフェニルアラニン、チロシン、トリプトファンといった芳香族アミノ酸に置換すると、芳香環とヌクレオシド塩基との間にπ-π相互作用が生じ、ヌクレオシドのＥＢ－ＡＰに対する親和性が向上することが予測された。同様に、バリン、ロイシン、イソロイシンといった分岐鎖疎水性アミノ酸に置換すると、分岐鎖疎水基とヌクレオシド塩基との間にＣＨ／π相互作用が生じ、また、グルタミン酸、アスパラギン酸といった負電荷を帯びたアミノ酸に置換すると、ヌクレオシド塩基の正電荷と静電的に引き合い、親和性の向上が見込まれた。そこで、ホスファターゼ活性よりもリン酸基転移活

性が相対的に高まっているＧ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰのリン酸基転移活性を更に高めるため、該変異型ＥＢ－ＡＰのＳ７２Ｆ，Ｓ７２Ｙ，Ｓ７２Ｗ，Ｓ７２Ｖ，Ｓ７２Ｅ，Ｓ７２Ｄ変異体を作製することとした。なお、Ｓ７２を他のアミノ酸に置換した変異体も作製することとした。ちなみに、これら変異体は、３残基置換変異型ＥＢ－ＡＰとなる。

【００２８】実施例１０　Ser７２を他のアミノ酸へ置換した３残基置換変異型ＥＢ－ＡＰの作製

エシェリヒア・コリJM１０９で発現するための９種類の変異型ＥＢ－ＡＰを構築するために、Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰ遺伝子を含むプラスミドpEPI３４０を、ＰＣＲを用いる部位特異的突然変異誘発法の鋳型として使用した。なお、pEPI３４０は、プラスミドpEPI３０５にＧ７４Ｄ／Ｉ１５３Ｔの変異を加えたプラスミドである。しかして、これらプラスミドpEPI３０５及びpEPI３４０の塩基配列は、特開平１０－２０１４８１号公報の段落番号（０１４３）の表１２に明記されている。また、プラスミドpEPI３０５をエシェリヒア・コリJM１０９に保持させた株は、AJ１３１４４と命名され、通商産業省工業技術院生命工学工業技術研究所にＦＥＲＭ　ＢＰ－５４２３として国際寄託されている〔上記公開公報の段落番号（０１０５）～（０１１０）の記載参照〕。変異はストラタジーン（Stratagene）社（アメリカ）の「クイックチェンジ部位特異的突然変異誘発キット（Quickchange Site-Directed Mutagenesis Kit）」を使用し、製造元のプロトコールに従って、各種変異型酵素に対応するプライマー（図７、配列表の配列番号１１～６１）を用いて導入した。ＰＣＲ反応の生成物を用いて、エシェリヒア・コリＸＬ－１を形質転換した。形質転換細胞を、１００μｌ／ｍｌのアンピシリンを含むＬ寒天培地プレート上に塗沫し、３７℃で１６時間インキュベートした。生成したコロニーを採取し、１００μｌ／ｍｌのアンピシリンを含むＬ培地で一晩振とうしながら培養した。培養液から遠心分離により菌体を回収後、ファルマシア社（スウェーデン）のFlexiPrep Kitを使用し、製造元のプロトコールに従って、プラスミドの抽出を行った。各種３残基置換変異型ＥＢ－ＡＰをコードする塩基配列は、ＤＮＡ配列分析によって確認した。なお、図７に示したプライマーセットの合成は(株)日本バイオサービスに委託した。

【００２９】実施例１１　変異型ＥＢ－ＡＰのリン酸基転移活性及び反応速度定数の測定

各種３残基置換変異型ＥＢ－ＡＰ遺伝子を含むプラスミドを導入したエシェリヒア・コリJM１０９を１００μｌ／ｍｌのアンピシリンを含むＬ培地５０ｍｌに接種し、３７℃で１６時間培養した。培養液から遠心分離で菌体を集め、２５ｍＭリン酸バッファー（ｐＨ７．０）３ｍｌに懸濁し、４℃で２０分間超音波処理を行い破砕した。処理液を遠心分離して不溶性画分を除き、無細胞抽出液を調製した。各ＥＢ－ＡＰ３残基置換変異型酵素が発現していることは、ＳＤＳ－ＰＡＧＥで確認した。発現量は、全蛋白質の２０％程度であった。無細胞抽出液のリン酸基転移活性は以下の条件で測定した。２ｍＭイノシン、１００ｍＭピロリン酸ナトリウム、１００ｍＭ酢酸バッファー（ｐＨ４．０）、１００μｌの無細胞抽出液を含む反応液（１ｍｌ）を、ｐＨ４、３０℃で１０分間インキュベートした。１Ｎ塩酸２００μｌを加え反応を停止させた後、遠心分離により沈殿を除き、生成した５′－イノシン酸を定量した。各種３残基置換変異型ＥＢ－ＡＰのリン酸基転移活性を、変異を導入する対象としたＧ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰを用いたときの５′－イノシン酸生成量を１とした相対活性で示した。

続いて、各種３残基置換変異型ＥＢ－ＡＰのリン酸基転移反応におけるイノシンに対するＫｍ値を以下の条件で測定した。１００ｍＭピロリン酸ナトリウム、１００ｍＭ酢酸バッファー（ｐＨ４．０）、１０－１００ｍＭイノシン、１００μｌの無細胞抽出液を含む反応液（１ｍｌ）をｐＨ４、３０℃で１０分間インキュベートした。１Ｎ塩酸２００μｌを加え反応を停止させた後、遠心分離により沈殿を除き、生成した５′－イノシン酸を定量した。Hanes-Woolfプロットにより Ｋｍ値を算出した。表２に結果を示す。

【００３０】

【表２】

	Km値	リン酸転移活性
S72F/G74D/I153T	20mM	2.80
S72Y/G74D/I153T	25mM	2.04
S72W/G74D/I153T	30mM	1.71
S72D/G74D/I153T	33mM	1.59
S72V/G74D/I153T	40mM	2.46
S72E/G74D/I153T	40mM	3.19
S72M/G74D/I153T	46mM	1.94
S72T/G74D/I153T	50mM	1.91
S72L/G74D/I153T	57mM	2.24
S72R/G74D/I153T	59mM	1.99
S72Q/G74D/I153T	77mM	2.42
S72K/G74D/I153T	78mM	1.53
S72P/G74D/I153T	109mM	1.34
S72A/G74D/I153T	115mM	0.78
S72N/G74D/I153T	124mM	0.43
S72G/G74D/I153T	137mM	0.43
S72H/G74D/I153T	n.d.	n.d.
G74D/I153T	100mM	1.00
10残基置換変異型	40mM	1.44

【００３１】実施例９にて、π-π相互作用、ＣＨ／π相互作用、静電相互作用によりイノシンとの親和性が向上するであろうと予測されたすべての変異体(Ｓ７２Ｆ,Ｓ７２Ｙ,Ｓ７２Ｗ,Ｓ７２Ｖ, Ｓ７２Ｌ,Ｓ７２Ｅ,Ｓ７２Ｄ)のイノシンに対するＫｍ値が、変異導入しないＧ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰのものに比べて低下し、イノシンに対する親和性が向上した。また、リン酸基転移活性についても向上が見られた。特に、Ｓ７２Ｆを導入した変異体が、Ｋｍ値、リン酸基転移活性、双方において、改善が著しかった。フェニルアラニンの芳香環とイノシン塩基が適当な位置関係でπ－π相互作用し、親和性の向上が図られたものと推測される。また、Ｓ７２Ｍ,Ｓ７２Ｔ,Ｓ７２Ｒ,Ｓ７２Ｑ,Ｓ７２Ｋ変異体のＫｍ値も低下した。これらアミノ酸残基とヌクレオシド塩基の間に疎水性相互作用、水素結合等何らかの好ましい相互作用が生じたものと考えられる。ちなみに、Ｓ７２Ｉについては、遺伝子を作製できなかった。また、Ｓ７２Ｃは、誤ったＳ－Ｓ結合を形成させる危険性があるので、作製しなかった。なお、5′－イノシン酸は、高速液体クロマトグラフィー(HPLC)により、下記の条件にて分析した。

カラム：Cosmosil ５Ｃ１８-ＡＲ(４．６×１５０mm)
ナカライテスク社製品

移動相：５ｍＭ　リン酸カリウムバッファー(ｐＨ２．８)／メタノール＝９５／５

流速：１．０ｍｌ／ｍｉｎ

温度：室温

検出：ＵＶ２４５ｎｍ

【００３２】実施例１２　Ｓ７２Ｆ／Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰ遺伝子を導入したエシェリヒア・コリJM１０９を用いた5′-イノシン酸の生産

Ｇ７４Ｄ／Ｉ１５３Ｔ変異型、１０残基置換変異型、及びＳ７２Ｆ／Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰ遺伝子を含むプラスミドを導入したエシェリヒア・コリJM１０９をアンピシリン１００μｇ／ｍｌ及びＩＰＴＧ1mMを含むＬ培地５０ｍｌに接種し、３７℃で１６時間培養した。ピロリン酸１２ｇ／ｄｌ、及びイノシン６ｇ／ｄｌを酢酸バッファー（ｐＨ４．０）に溶解し、これに上記の各変異型ＥＢ－ＡＰ遺伝子を導入したエシェリヒア・コリJM１０９の菌体を乾燥菌体重量で１００ｍｇ／ｄｌとなるように添加し、ｐＨを４．０に維持しながら、３０℃で２４時間反応を行った。生成した5′－イノシン酸の量を測定した結果を表３に示した。なお、生成したイノシン酸は5′－イノシン酸のみで2′－イノシン酸及び3′－イノシン酸の副生は全く認められなかった。Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰ遺伝子を含むプラスミドを導入したエシェリヒア・コリJM１０９を用いた反応では７．５ｇ／ｄｌの5′－イノシン酸が生成蓄積したが、反応時間を伸ばしてもそれ以上蓄積は増加しなかった。１０残基置換変異型ＥＢ－ＡＰ遺伝子を含むプラスミドを導入したエシェリヒア・コリJM１０９を用いた反応では蓄積が向上し、１２．１ｇ／ｄｌの5′-イノシン酸が生成蓄積した。立体構造に基づいて設計し、構築したＳ７２Ｆ／Ｇ７４Ｄ/Ｉ１５３Ｔ変異型ＥＢ－ＡＰ遺伝子を含むプラスミドを導入したエシェリヒア・コリJM１０９を用いた反応では更に生産性が向上し、１３．２ｇ／ｄｌの5′－イノシン酸が生成蓄積した。

【００３３】

【表３】

導入した変異型酵素遺伝子	生成イノシン酸(g/dl)
G74D/I153T	7.5
10残基置換変異型	12.1
S72F/G74D/I153T	13.2

【００３４】実施例１３　Ｌ１６Ｗ,Ｓ７１Ｗ,Ｓ７３Ｗ,Ｅ１０４Ｆ,Ｅ１０４Ｗ変異を導入した３残基置換変異型ＥＢ－ＡＰのリン酸基転移活性及び反応速度定数の測定

Ｓ７２Ｆ変異が、π－π相互作用によりイノシンとの親和性を向上させたと考えられるため、コンピューターグラフィックス上で、芳香環アミノ酸への置換によりイノシン塩基とのπ－π相互作用が図れる他のアミノ酸残基を探索した。その結果、Ｌ１６Ｗ,Ｓ７１Ｗ,Ｓ７３Ｗ,Ｅ１０４Ｆ,Ｅ１０４Ｗ変異により置換された芳香環がイノシン塩基と相互作用する可能性が示唆された。そこで、これら５種の（Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰをベースとした）３残基置換変異型ＥＢ－ＡＰを実施例１０に記述した方法（各変異型酵素に対応するプライマーは図８に示した。配列表の配列番号６２～７６）で作製し、実施例１１に記述した方法でリン酸基転移活性及び反応速度定数を測定した。結果を表４に示す。リン酸基転移活性はいずれの変異型酵素においても低下したが、Ｋm値はすべての変異型酵素で低下し、イノシンとの親和性が向上したことが示唆された。Leu１６は、イノシンとの相互作用が確実視されるSer７２から（Ｃα間距離で）１０Å離れているが、この程度離れていてもイノシンとの相互作用が可能であることが示された。なお、図８に示したプライマーセットの合成は、(株)日本バイオサービスに委託した。

【００３５】

【表４】

	Km値	リン酸基転移活性
L16W/G74D/I153T	33mM	0.21
S71W/G74D/I153T	75mM	0.26
S73W/G74D/I153T	29mM	0.77
E104F/G74D/I153T	61mM	0.65
E104W/G74D/I153T	67mM	0.26
G74D/I153T	100mM	1.00
10残基置換変異型酵素	40mM	1.44

【００３６】実施例１４　エンテロバクター・アエロゲネス(Enterobacter aerogenes)由来酸性ホスファターゼ（ＥＡ－ＡＰ)の変異型酵素遺伝子の作製と該遺伝子を導入したエシェリヒア・コリJM１０９を用いた5′－イノシン酸の生産

ＥＢ－ＡＰにおいてイノシンのリン酸基転移活性を向上させたＳ７２Ｆ／Ｇ７４Ｄ／Ｉ１５３Ｔの３つの変異に相同な変異をＥＡ－ＡＰに導入することとした。ＥＢ－ＡＰとＥＡ－ＡＰのアミノ酸配列をプログラムBLASTを用いて、アラインメントした結果を図９に示す。ＥＢ－ＡＰのSer７２／Gly７４／Ile１５３は、ＥＡ－ＡＰにおいては、Ala７０／Gly７２／Ile１５１に対応することが示された。そこで、Ａ７０Ｆ／Ｇ７２Ｄ／Ｉ１５１Ｔ変異型ＥＡ-ＡＰを実施例１０に記述した方法で作製した。変異型酵素遺伝子を含むプラスミドを導入したエシェリヒア・コリJM１０９を用いて、イノシンから5′－イノシン酸の生産を実施例１２に記述した方法で行った。結果を表５に示す。Ａ７０Ｆ／Ｇ７２Ｄ／Ｉ１５１Ｔ変異型ＥＡ-ＡＰは、Ｓ７２Ｆ／Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰと同等の5′－イノシン酸生産能を示した。

【００３７】

【表５】

導入した変異型酵素遺伝子	生成イノシン酸(g/dl)
EA-AP A72F/G74D/I153T	13.1
EB-AP S72F/G74D/I153T	13.2

【００３８】なお、図９はＥＢ－ＡＰとエンテロバクター・アエロゲネス由来酸性ホスファターゼ（ＥＡ－ＡＰ）のアミノ酸配列アラインメントをプログラムBLASTにより行った結果を示す図である。上段がＥＢ－ＡＰ、下段がＥＡ－ＡＰである。中段には、両者のアミノ酸残基が同一であればその残基名が、同一でなくても類似のアミノ酸残基であれば、＋が表示される。ＥＢ－ＡＰの７２番目の残基（Ser７２）の位置を、［７２］でマークした。ＥＡ－ＡＰで対応する残基はAla７０である。

【００３９】実施例１５　モルガネラ・モルガニ由来酸性ホスファターゼ（ＭＭ－ＡＰ）由来Ｇ７２Ｄ／Ｉ１５１Ｔ変異型酵素の結晶化及び結晶構造解析

ハンギングドロップ法での蒸気拡散を利用してＭＭ－ＡＰのG72D/I151T二重変異体の結晶化を行った。当該蛋白質溶液（濃度40mg/ml）と、25（w/v%）のポリエチレングリコール１０００、25mM 硫安、25mM DTTを含む125mM クエン酸緩衝液（pH4.8)を同量ずつ（各々5μｌ）、シリコナイゼイションしたカバーグラス上に滴下混合し、25（w/v%）のポリエチレングリコール１０００、25mM 硫安、25mM DTTを含む125mM クエン酸緩衝液（pH4.8)500μlを満たしたウエルの上に混合液滴が釣り下がるようにかぶせ、20℃にて静置した。２、３日後に結晶が析出し、１週間後には測定可能な大きさ（0.4×0.4×0.3mm程度）に成長した。（株）リガクのＸ線回折装置R-AXIS IIcを用いて、Ｘ線回折データを収集し、結晶学的パラメーターを決定した。空間群は$P2_12_12_1$、格子定数はa＝90.64Å、b＝119.74Å、c＝136.14Åとなった。筑波・高エネルギー研究所シンクロトロン放射光施設BL-6B上、100Kで、2.6Å分解能データまでの回折データを測定した。単位格子の体積、空間群、酵素の分子量から見積もって、非対称単位には６量体の分子１つが含まれることが予想された。そこで、野生型ＥＢ－ＡＰの６量体構造を探索モデルとして、プログラムamoreを用いて分子置換法により解析を行った。rotation searchにおいては１０～３Å分解能のデータを、translation searchにおいては１０～４Å分解能のデータを用いた。両サーチともに、正解がトップピークとして現れた。分子を剛体として精密化を行った後、QUANTAを用いたグラフィックス上での構造修正とX-PLORを用いた構造精密化を繰り返し行い、１０～2.6Å分解能において、Ｒ因子0.197のモデルを得た。図１に示した５つの活性残基（Ｌｙｓ１１３、Ａｒｇ１２０、Ｈｉｓ１４８、Ａｒｇ１８１、Ｈｉｓ１８７）のＣα原子間の距離を表６に示した。ＥＢ－ＡＰの類縁酵素であるＭＭ－ＡＰにおいて、活性残基間のすべての原子間距離が、ＥＢ－ＡＰの立体構造から規定した範囲におさまっていることが確認された。

【００４０】

【表６】

		G74D/I153T 変異型MM-AP	下限	上限
Lys113	Arg120	11.3Å	10.4Å	12.6Å
	His148	12.6Å	11.3Å	13.8Å
	Arg181	16.3Å	14.5Å	17.4Å
	His187	12.5Å	10.7Å	13.6Å
Arg120	His148	14.0Å	12.2Å	15.2Å
	Arg181	10.9Å	9.4Å	11.8Å
	His187	6.1Å	4.5Å	6.7Å
His148	Arg181	8.9Å	6.7Å	9.8Å
	His187	10.2Å	8.8Å	11.0Å
Arg181	His187	5.4Å	4.5Å	6.8Å

【００４１】実施例１６　Ａ７２Ｆ、Ａ７２Ｅ変異を導入した１０残基置換変異型ＥＢ－ＡＰの作製、及びリン酸転移活性と反応速度定数の測定

実施例１１において、最もＫｍ値を低下させたＳ７２Ｆと最も活性を高めたＳ７２Ｅ変異を、１０残基置換変異型ＥＢ－ＡＰに導入した。１０残基置換変異型ＥＢ－ＡＰにおいては、Ｓｅｒ７２がＡｌａに置換されているので、実際には、Ａ７２ＦとＡ７２Ｅ変異を導入することとなる。野生型ＥＢ－ＡＰを基準とすると、双方とも１０残基が置換されている。これら２種の変異体を実施例１０に記述した方法（各変異型酵素に対応するプライマーは図４６に示した。配列表の配列番号７７～８２）で作製した。ＰＣＲを用いる部位特異的突然変異誘発法の鋳型としては、１０残基置換変異型ＥＢ－ＡＰ遺伝子を含むプラスミドpEMP370（特開平9-37785号公報、実施例１９）を使用した。更に、実施例１１に記述した方法でリン酸転移活性及び反応速度定数を測定した。結果を表７に示す。リン酸転移活性は、Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰを用いたときの５′－イノシン酸生成量を１とした相対活性で示した。いずれの変異型酵素においても、Ｋｍ値は顕著に低下した。リン酸転移活性については、Ａ７２Ｆ変異により低下したのに対し、Ａ７２Ｅ変異により上昇した。

【００４２】

【表７】

	Km値	リン酸転移活性
A72F/10残基置換変異型酵素	9mM	0.11
A72E/10残基置換変異型酵素	15mM	2.30
10残基置換変異型酵素	40mM	1.44

【００４３】実施例１７　Ａ７２Ｆ／１０残基置換変異型ＥＢ－ＡＰ及びＡ７２Ｅ／１０残基置換変異型遺伝子を導入したエシェリヒア・コリＪＭ109を用いた５′－イノシン酸の生産

Ａ７２Ｆ／１０残基置換変異型ＥＢ－ＡＰ及びＡ７２Ｅ／１０残基置換変異型遺伝子を含むプラスミドを導入し

たエシェリヒア・コリＪＭ109を用いての５′－イノシン酸の生産実験を実施例１２に記述した方法で行った。結果を表８に示す。両変異体とも５′－イノシン酸の蓄積量が増加した。

【００４４】

【表８】

導入した変異型酵素遺伝子	生成イノシン酸(g/dl)
A72F/10残基置換変異型	13.9
A72E/10残基置換変異型	13.9
10残基置換変異型	12.1

【００４５】実施例１８　Ｉ１０３Ｄ、Ｔ１５３Ｎ変異を導入した変異型ＥＢ－ＡＰの作製、及びリン酸転移活性及び反応速度定数の測定

Ｉ１０３Ｄ変異により、置換されたＡｓｐがイノシン塩基と静電相互作用をすること、また、Ｔ１５３Ｎ変異により、置換されたＡｓｎがリボースの水酸基と水素結合を形成することが図３のモデルにより示唆された。そこで、Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ--ＡＰにこれらの残基を導入し、Ｉ１０３Ｄ／Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰとＧ７４Ｄ／Ｉ１５３Ｎ変異型ＥＢ－ＡＰを実施例１０に記述した方法（各変異型酵素に対応するプライマーは図４７に示した。配列表の配列番号８３～８８）で作製することとした。更に、実施例１１に記述した方法でリン酸転移活性及び反応速度定数を測定した。結果を表９に示す。リン酸転移活性は、Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰを用いたときの５′－イノシン酸生成量を１とした相対活性で示した。両変異体ともに、リン酸基転移活性は低下したが、Ｋｍ値は低下し、イノシンとの親和性が向上したことが示唆された。

【００４６】

【表９】

	Km値	リン酸転移活性
I103D/G74D/I153T	51mM	0.09
G74D/I153N	38mM	0.18
G74D/I153T	100mM	1.00

【００４７】実施例１９ Leu140をＰｈｅ、Ｇｌｕ、Ｌｙｓに置換した変異型ＥＢ－ＡＰの作製、及びリン酸転移活性と反応速度定数の測定

Leu140は、Ser72から１０Å以上離れているが、反応中間体アナログの立体構造において、リン酸結合部位の直近に位置する。したがって、この残基を置換すれば、反応中間体におけるリン酸結合部位周辺の構造が変化し、ひいては、ヌクレオシド結合部位の構造と揺らぎにも影響が及ぶものと考えられた。この残基を、よりかさ高いＰｈｅ、正電荷を有するＬｙｓ、負電荷を有するＧｌｕ、に置換すれば、ヌクレオシドとの親和性が変化するのではないかと期待される。変異は、実施例１６においてリン酸転移活性が高かったＡ７２Ｅ／１０残基置換体に導入することとした。これら３種の変異体を実施例１０に記述した方法（各変異型酵素に対応するプライマーは図４８に示した。配列表の配列番号８９～９７）で作製した。ＰＣＲを用いる部位特異的突然変異誘発法の鋳型としては、Ａ７２Ｅ／１０残基置換変異型ＥＢ－ＡＰ遺伝子を含むプラスミドを使用した。更に、実施例１１に記述した方法でリン酸転移活性及び反応速度定数を測定した。結果を表１０に示す。リン酸転移活性は、Ｇ７４Ｄ／Ｉ１５３Ｔ変異型ＥＢ－ＡＰを用いたときの５′-イノシン酸生成量を１とした相対活性で示した。

【００４８】

【表１０】

	Km値	リン酸転移活性
A72E/L140F/10残基置換変異型酵素	9mM	1.66
A72E/L140K/10残基置換変異型酵素	78mM	0.07
A72E/L140E/10残基置換変異型酵素	322mM	0.16
A72E/10残基置換変異型酵素	15mM	2.30

【００４９】Ｌ１４０Ｆを導入した変異体は、Ｋｍ値が低下した。逆に、Ｌ１４０Ｋ及びＬ１４０Ｅ変異は、Ｋｍを大幅に上昇させた。

【００５０】実施例２０　エンテロバクター・アエロゲネス IF0 12010 由来野生型酸性ホスファターゼの精製とＮ末端アミノ酸配列の決定

特開平10-201481号公報の実施例24記載のエシェリヒア・コリJM109/pENP110の培養菌体からエンテロバクター・アエロゲネスIF012010由来の酸性ホスファターゼを精製してＮ末端アミノ酸配列を決定し、成熟蛋白質のアミノ酸配列を決定した。エシェリヒア・コリ JM109/pENP110はエンテロバクター・アエロゲネスIF012010由来の酸性ホスファターゼ遺伝子をエシェリヒア・コリJM109株に導入した菌で、該酸性ホスファターゼを生産する。該

酸性ホスファターゼ遺伝子の塩基配列より予想される前駆体蛋白質のアミノ酸配列は配列表の配列番号１０に示される配列である。ペプトン１g/dl、酵母エキス0.5g/dl及び食塩１g/dlを含有する栄養培地（pH7.0）50mlを500ml坂口フラスコに入れ、120℃にて20分間加熱殺菌した。これにエシェリヒア・コリ JM109/pENP110を一白金耳接種し、30℃で16時間振とう培養した。培養液から遠心分離により菌体を回収した菌体を100 ｍｌの100mMリン酸カリウムバッファー（pH7.0）に懸濁し、４℃で20分間超音波処理を行い菌体を破砕した。処理液を遠心分離して不溶性画分を除き、無細胞抽出液を調製した。この無細胞抽出液に30%飽和となるように硫酸アンモニウムを添加した。遠心分離により生成した沈殿を除去した後、上清液に60%飽和となるように硫酸アンモニウムを追加添加した。生成した沈殿を遠心分離により回収し、100mMリン酸カリウムバッファーに溶解した。この粗酵素液を100mMリン酸カリウムバッファー（pH7.0）500mlに対し3回透析した後、20mMリン酸カリウムバッファー（pH7.0）で平衡化したDEAE-トヨパール650Mカラム（φ3.0×10.0cm）にチャージし、20mMリン酸カリウムバッファー（pH7.0）で洗浄した。リン酸転移活性は素通り画分にあったので、当該画分を回収した。 この活性画分に35%飽和となるように硫酸アンモニウムを添加し、これを35%飽和硫酸アンモニウムを含む20mMリン酸カリウムバッファー（pH7.0）で平衡化したブチルトヨパールカラム（φ3.0×7.0cm）に吸着させた。これを35%飽和から20%飽和リン酸カリウムバッファー（pH7.0）の直線的な濃度勾配で溶出した。活性画分を集め、10mMリン酸カリウムバッファー（pH6.0）１Ｌに対し透析した後、10mMリン酸カリウムバッファー（pH6.0）で平衡化したCM-トヨパール カラム（φ3.0×7.0cm）に吸着させた。これを0mMから300mM 塩化カリウムを含むリン酸カリウムバッファー（pH6.0）の直線的な濃度勾配で溶出した。この活性画分を集めた。以上の操作によって、リン酸転移活性を示す酵素を無細胞抽出液より最終的に約16%の回収率で約5倍に精製した。この酵素標品は、ＳＤＳ－ポリアクリルアミド電気泳動において均一であった。本精製酵素をＤＩＴＣ メンブレン〔ミリゲン／バイオサーチ（Milligen/Biosearch）社製〕に吸着させ、Prosequencer 6625（ミリゲン／バイオサーチ社製）を用いてＮ末端のアミノ酸配列を決定したところ配列表の配列番号９８に示した５残基のＮ末端のアミノ酸配列が決定された。精製酵素のＮ末端は配列表の配列番号１０の配列の21番目のアラニン残基から開始していたため、配列表の配列番号１０に示されるアミノ酸配列は前駆体蛋白質の配列であり、１番目のメチオニン残基から20番目のフェニルアラニン残基までのペプチドは翻訳後に除去されるものと考えられた。この結果より成熟体蛋白質のアミノ酸配列は配列表の配列番号１０に示される配列中、アミノ酸番号１～２２８に示される配列であると考えられた。

【００５１】実施例２１　エンテロバクター・アエロゲネス IFO 12010 由来新規変異型酸性ホスファターゼ遺伝子のプロモーター配列の改変による酵素の高発現
エンテロバクター・アエロゲネス IFO 12010由来変異型酸性ホスファターゼをコードする遺伝子のプロモーター配列部分に遺伝子工学的手法によって部位特異的変異を導入し、酵素発現量の増加した変異型酸性ホスファターゼをコードする遺伝子を構築した。変異を導入する遺伝子は特願平12-189226号明細書の実施例3にて構築したプラスミドpENP170を用いた。本プラスミドはエンテロバクター・アエロゲネス IFO 12010由来変異型酸性ホスファターゼをコードする遺伝子を含む制限酵素ＳａｌＩと制限酵素ＫｐｎＩで切り出される1.6kbpＤＮＡ断片を、ＳａｌＩ及びＫｐｎＩで切断したpUC19（宝酒造社製）に連結したプラスミドＤＮＡであり、pENP170中のＳａｌＩ－ＫｐｎＩ 1.6kbpＤＮＡ断片の塩基配列は配列表の配列番号９に示される配列である。プラスミドＤＮＡへの変異導入はストラタジーン社製のクイックチェンジ部位特異的突然変異誘発キット（Quick Change site-directed mutagenesis kit）を用いた。ＤＮＡ合成装置（アプライドバイオシステム社製モデル394）を用いて合成した変異導入用オリゴヌクレオチドMUT170（配列表の配列番号９９）、MUT171（配列表の配列番号１００）、及び鋳型としてpENP170を用いてストラタジーン社のプロトコールに従って変異を導入した。得られたプラスミドＤＮＡを用いて常法によりエシェリヒア・コリＪＭ109（宝酒造社製）を形質転換した。これを100μg/mlのアンピシリンを含むＬ寒天培地上にプレーテイングし、形質転換体を得た。形質転換体よりアルカリ溶菌法によりプラスミドを調製し、塩基配列の決定を行い、目的の塩基が置換されていることを確認した。塩基配列の決定は Taq DyeDeoxy Terminator Cycle Sequencing Kit （アプライドバイオケミカル社製）を用い、サンガーらの方法〔ジャーナル オブ モレキュー バイオロジー（J. Mol. Biol.）、第143巻、第161頁（1980）〕に従って行った。このようにしてエンテロバクター・アエロゲネス IFO 12010由来推定酸性ホスファターゼの上流に位置する推定プロモーター配列の－１０領域の塩基配列がAAAAATからエシェリヒア・コリのlacプロモーターと同じTATAATという塩基配列に変異した変異型遺伝子をコードする変異型遺伝子を構築した。この変異型遺伝子を含むプラスミドをpENP180と命名した。

【００５２】エシェリヒア・コリ ＪＭ109／pENP170及びプロモーター配列の－１０領域を改変した遺伝子を導入したエシェリヒア・コリＪＭ109／pENP180をアンピシリン100μg/mlを含むＬ培地50ｍｌ、及びＩＰＴＧ1mMを添加したアンピシリン100μg/mlを含むＬ培地50ｍｌにそれぞれ接種し、37℃で16時間培養した。それぞれの菌の培養液から遠心分離により菌体を集め、生理食塩水

で1回洗浄した。ピロリン酸15g/dl、及びイノシン、8g/dlを100ｍＭ酢酸バッファー（pH4.0）に溶解し、これにそれぞれの菌体を乾燥菌体重量で100mg/dlとなるように添加し、pHを4.0に維持しながら、30℃で1時間反応させた。生成した5′－イノシン酸の量を表１１に示した。イノシン及び５′－イノシン酸は、高速液体クロマトグラフィー（ＨＰＬＣ）により、下記の条件にて分析した。

カラム：Cosmosil 5C18-AR （4.6×150mm）〔ナカライテスク社製品〕

移動相：5mM リン酸カリウムバッファー（pH 2.8）／メタノール ＝ 95／5

流速：1.0ml/min

温度：室温

検出：ＵＶ245nm

エシェリヒア・コリＪＭ109／pENP170ではIPTG無添加では活性が低かったが、エシェリヒア・コリＪＭ109／pENP180はIPTGを添加しなくても高い活性を示した。また、エシェリヒア・コリＪＭ109／pENP180はIPTGを添加することでさらに高い活性を発現し、プロモーター領域の改変が有効であることが示された。

【００５３】

【表１１】

菌　株	IPTG	生成5′－イノシン酸(g/dl)
エシェリヒア・コリ JM109/pENP170	無添加	０．７３
	１mM添加	３．０９
エシェリヒア・コリ JM109/pENP180	無添加	２．８６
	１mM添加	３．３７

【００５４】実施例２２　ヌクレオシドに対する親和性の向上したエンテロバクター・アエロゲネス IFO 12010由来新規変異型酸性ホスファターゼ遺伝子の構築

実施例２１にて構築したエンテロバクター・アエロゲネス IFO 12010由来変異型酸性ホスファターゼ遺伝子に遺伝子工学的手法によって部位特異的変異を導入し、ヌクレオシド、特にグアノシンに対する親和性が向上した変異型酸性ホスファターゼをコードする遺伝子を作製した。アミノ酸残基の置換はエシェリヒア・ブラッタエ酵素の立体構造解析に基づいてヌクレオシドとの親和性向上に寄与すると同定されたアミノ酸残基の置換を組合せて導入した。プラスミドＤＮＡへの変異導入はストラタジーン社製のクイックチェンジ部位特異的突然変異誘発キット（Quick Change site-directed mutagenesis kit）を用いた。ＤＮＡ合成装置（アプライドバイオシステム社製モデル394）を用いてMUT180（配列表の配列番号１０１）からMUT521（配列表の配列番号１２０）までの20種類の変異導入用オリゴヌクレオチドを合成した。最初の鋳型としてpENP170、また変異導入用オリゴヌクレオチドとしてMUT180、MUT181を用いてストラタジーン社のプロトコールに従って変異を導入した。得られたプラスミドＤＮＡを用いて常法によりエシェリヒア・コリＪＭ109（宝酒造社製）を形質転換した。これを100μg/mlのアンピシリンを含むＬ寒天培地上にプレーティングし、形質転換体を得た。形質転換体よりアルカリ溶菌法によりプラスミドを調製し、塩基配列の決定を行い、目的の塩基が置換されていることを確認した。塩基配列の決定は Taq DyeDeoxy Terminator Cycle Sequencing Kit （アプライドバイオケミカル社製）を用い、サンガーらの方法（前出の学会誌）に従って行った。このようにして153番目のスレオニン残基（ACC）がセリン残基（TCC）に置換した変異型酸性ホスファターゼをコードする遺伝子を構築し、この変異型遺伝子を含むプラスミドをpENP200と命名した。変異を導入したプラスミドを新しい鋳型として同様の操作を繰り返し、累加的に部位特異的変異を導入した。形質転換体よりアルカリ溶菌法によりプラスミドを調製し、塩基配列の決定を行い、目的の塩基が置換されていることを確認した。作成した変異型酸性ホスファターゼをコードする変異型酵素遺伝子と変異部位を表１２に示した。なお変異部位のアミノ酸残基は配列表の配列番号１０に示した成熟蛋白質のアミノ酸配列中のアミノ酸残基を示している。

【００５５】それぞれの変異型酸性ホスファターゼ遺伝子を含むプラスミドを導入したエシェリヒア・コリ ＪＭ109／pENP180、エシェリヒア・コリＪＭ109／pENP320、エシェリヒア・コリＪＭ109／pENP340、エシェリヒア・コリＪＭ109／pENP410、エシェリヒア・コリＪＭ109／pENP510、及びエシェリヒア・コリＪＭ109／pENP520をアンピシリン100μg/ml及びＩＰＴＧ1mMを含むＬ培地50mｌに接種し、37℃で16時間培養した。菌体を50mｌの100ｍＭリン酸バッファー（pH7.0）に懸濁し、4℃で20分間超音波処理を行い菌体を破砕した。それぞれの菌の培養液から遠心分離により菌体を集め、生理食塩水で1回洗浄した。処理液を遠心分離して不溶性画分を除き、無細胞抽出液を調製した。それぞれの無細胞抽出液を用いてリン酸転移反応におけるイノシンとグアノシンに対するKm値を測定した。

【００５６】ヌクレオシドへのリン酸転移活性の測定は、イノシン及びグアノシンを基質として次の条件で行った。各種濃度のイノシン又はグアノシン、ピロリン酸ナトリウム100μmol/ml、酢酸ナトリウム緩衝液（pH4.0）100μmol/ml及び酵素を含む反応液（1ml）でpH4.0、

30℃で10分反応を行った。2N塩酸200μlを添加して反応を停止した後、遠心分離により沈殿を除き、リン酸転移反応により生成した5′－イノシン酸又は5′－グアニル酸を定量した。イノシン、グアノシン、5′－イノシン酸及び5′－グアニル酸は、高速液体クロマトグラフィー（ＨＰＬＣ）により、実施例２１と同じ条件にて分析した。上記の組成の反応条件においてイノシン又はグアノシンの濃度を変化させてリン酸転移活性を測定し、Hanes-Woolfプロット〔ザ バイオケミカル ジャーナル（Biochem. J.）、第26巻、第1406頁(1932)〕によりリン酸転移反応におけるイノシン及びグアノシンの速度定数を求めた。その結果を表１３～表１６に示した。実施例で作成した変異型酵素のKm値はグアノシンに対するＫｍ値が顕著に低下し、グアノシンに対する親和性が向上していることが明らかになった。またpENP520にコードされる変異型酵素以外の４種類の変異型酵素はイノシンに対するＫｍ値も非常に低下していた。

【００５７】

【表１２】

配列の名称	配列番号		長さ	配　列
MUT170	99	センス	30	5′-CTT ACA GAT GAC **TAT AAT** GTG ACT AAA AAC
MUT171	100	アンチセンス	30	5′-GTT TTT AGT CAC ATT ATA GTC ATC TGT AAG
MUT180	101	センス	33	5′-TCT ACC GGT TGG GCA **TCC** GCG CTG GTA CTG GCG
MUT181	102	アンチセンス	33	5′-CGC CAG TAC CAG CGC GGA TGC CCA ACC GGT AGA
MUT300	103	センス	33	5′-TCC GGC CAT ACC TCT **TCC** GGT TGG GCA TCC GCG
MUT301	104	アンチセンス	33	5′-CGC GGA TGC CCA ACC GGA AGA GGT ATG GCC GGA
MUT310	105	センス	33	5′-GAT GCT GAC CTG GCC **GTT** GGC GAC GTC GCG AAT
MUT311	106	アンチセンス	33	5′-ATT CGC GAC GTC GCC AAC GGC CAG GTC AGC ATC
MUT320	107	センス	33	5′-CTG ACA AAT ATG ATT **CTG** GAT GCC GGC GAT CTG
MUT321	108	アンチセンス	33	5′-CAG ATC GCC GGC ATC CAG AAT CAT ATT TGT CAG
MUT330	109	センス	33	5′-GAT GCT GAC CTG GCC **ATG** GGC GAC GTC GCG AAT
MUT331	110	アンチセンス	33	5′-ATT CGC GAC GTC GCC CAT GGC CAG GTC AGC ATC
MUT340	111	センス	33	5′-CTG ACA AAT ATG ATT **CAG** GAT GCC GGC GAT CTG
MUT341	112	アンチセンス	33	5′-CAG ATC GCC GGC ATC CTG AAT CAT ATT TGT CAG
MUT400	113	センス	33	5′-TCC GGC CAT ACC TCT **GCT** GGT TGG GCA TCC GCG
MUT401	114	アンチセンス	33	5′-CGC GGA TGC CCA ACC AGC AGA GGT ATG GCC GGA
MUT500	115	センス	33	5′-TCC GGC CAT ACC TCT **GGC** GGT TGG GCA TCC GCG
MUT501	116	アンチセンス	33	5′-CGC GGA TGC CCA ACC GCC AGA GGT ATG GCC GGA
MUT510	117	センス	33	5′-GAT GCT GAC CTG GCC **GAA** GGC GAC GTC GCG AAT
MUT511	118	アンチセンス	33	5′-ATT CGC GAC GTC GCC TTC GGC CAG GTC AGC ATC
MUT520	119	センス	33	5′-GAT GCT GAC CTG GCC **AAA** GGC GAC GTC GCG AAT
MUT521	120	アンチセンス	33	5′-ATT CGC GAC GTC GCC TTT GGC CAG GTC AGC ATC

【００５８】

【表１３】

プラスミド名	変異を導入したプラスミド	変異導入に用いたプライマー	変異点及びアミノ酸置換
pENP180			61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 151I(ATC)→T(ACC)
pENP200	pENP130	MUT180, MUT181	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 151T(ACC)→S(TCC)
pENP300	pENP200	MUT300, MUT301	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 151I(ATC)→T(ACC) 149T(ACC)→S(TCC) 151T(ACC)→S(TCC)
pENP310	pENP300	MUT310, MUT311	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 70A(GCC)→V(GTT) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 151I(ATC)→T(ACC) 149T(ACC)→S(TCC) 151T(ACC)→S(TCC)
pENP320	pENP310	MUT320, MUT321	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC)

【００５９】

【表１４】

			69S(AGC)→A(GCC) 70A(GCC)→V(GTT) 72G(GGC)→D(GAC) 102E(GAG)→L(CTG) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→S(TCC) 151T(ACC)→S(TCC)
pENP330	pENP300	MUT330, MUT331	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 70A(GCC)→M(ATG) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→S(TCC) 151T(ACC)→S(TCC)
pENP340	pENP330	MUT340, MUT341	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 70A(GCC)→V(GTT) 72G(GGC)→D(GAC) 102E(GAG)→Q(CAG) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→S(TCC) 151T(ACC)→S(TCC)
pENP400	pENP200	MUT400, MUT401	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→A(GCT) 151T(ACC)→S(TCC)
pENP410	pENP400	MUT310, MUT311	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 70A(GCC)→V(GTT) 72G(GGC)→D(GAC)

【００６０】

【表１５】

			133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→A(GCT) 151T(ACC)→S(TCC)
pENP500	pENP200	MUT500, MUT501	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→G(GGC) 151T(ACC)→S(TCC)
pENP510	pENP500	MUT510, MUT511	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 70A(GCC)→E(GAA) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→G(GGC) 151T(ACC)→S(TCC)
pENP520	pENP500	MUT520, MUT521	61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG) 64E(GAA)→A(GCA) 67N(AAC)→D(GAC) 69S(AGC)→A(GCC) 70A(GCC)→K(AAA) 72G(GGC)→D(GAC) 133T(ACC)→K(AAA) 134E(GAG)→D(GAC) 149T(ACC)→G(GGC) 151T(ACC)→S(TCC)

【００６１】　　【表１６】

	イノシンに対するKm値（mM）	イノシンを基質とした場合の相対活性	グアノシンに対するKm値（mM）	グアノシンを基質とした時の相対活性
pENP180	４０	１．０	４０	１．０
pENP320	１９	１．９	４．６	１．５
pENP340	１９	１．４	５．１	１．３
pENP410	１８	１．０	４．９	０．７０
pENP510	１７	０．５５	４．０	０．３９
pENP520	４６	０．６３	４．４	０．２１

【００６２】実施例２３　グアノシンに対する親和性の向上したエンテロバクター・アエロゲネス IFO 12010由来新規変異型酸性ホスファターゼ遺伝子導入菌によるグアノシンのリン酸化反応

それぞれの変異型酸性ホスファターゼ遺伝子を含むプラスミドを導入したエシェリヒア・コリ ＪＭ109／pENP180、エシェリヒア・コリＪＭ109／pENP320、エシェリヒア・コリＪＭ109／pENP340、エシェリヒア・コリＪＭ109／pENP410、エシェリヒア・コリＪＭ109／pENP510、及びエシェリヒア・コリＪＭ109／pENP520をアンピシリン100μg/ml及びＩＰＴＧ1mMを含むＬ培地50mＩに接種し、37℃で16時間培養した。ピロリン酸10g/dl、及び特開平12-189226号公報の実施例１と同様に調製した粉砕グアノシン6.6g/dlを100mＭ酢酸バッファー（pH4.5）に溶解し、これにそれぞれの菌体を乾燥菌体重量で100mg/dlとなるように添加し、pHを4.5に維持しながら、35℃で12時間反応させた。生成した5′－グアニル酸の量を表１７に示した。表１７に示すように変異型酵素を導入した菌はいずれも親株であるエシェリヒア・コリ ＪＭ109／pENP180に比べて生産性が向上し、高い収率で5′

ーグアニル酸を生成蓄積した。

【０００３】

【表１７】

菌　株	生成 5'-グアニル酸（g/dl）
エシェリヒア・コリ JM109/pENP180	９．９０
エシェリヒア・コリ JM109/pENP320	１０．４
エシェリヒア・コリ JM109/pENP340	１０．２
エシェリヒア・コリ JM109/pENP410	１１．１
エシェリヒア・コリ JM109/pENP510	１１．０
エシェリヒア・コリ JM109/pENP520	１０．５

【０００４】

【発明の効果】以上詳細に説明したように、本発明によれば、ヌクレオシドー５′ーリン酸生産能が向上した変異型ヌクレオシドー５′ーリン酸生産酵素、及びその製造方法が提供される。また、本発明によれば、ヌクレオシドー５′ーリン酸の製造方法に有用な、前記の変異型酵素をコードする遺伝子、該遺伝子を含む組換えＤＮＡ、該組換えＤＮＡを保有する微生物が提供される。更に、Ｘ線結晶構造解析技術により蛋白質の新規立体構造の解明に成功した。

【０００５】

【配列表】

<;110>; Ajinomoto Co., Inc. (味の素株式会社)
<;120>; 変異型ヌクレオシドー５′ーリン酸生産酵素
<;130>; 整理番号　１－０００８０４－１
<;141>; 2000-09-03
<;150>; JP 11-249545
<;151>; 1999-09-03
<;160>;
<;170>; PatentIn Ver. 2.0

【０００６】

<;210>; 1
<;211>; 1225
<;212>; DNA
<;213>; Escherichia blattae
<;220>;
<;221>; CDS
<;222>; (331)..(1077)
<;400>; 1

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ctgcaggcga aaggcaatgt ggtggccggt gagacggcac tctacgagat taaggataag 60
taactatcca ttattacagg taacagcatt gctcctgagt gtgatgtcat acctgagcgg 120
cgcgggggtt ccccgggccg ctttttttta tggggctgcg gtgaggagcg ttatctgctg 180
gccctgtttg tgcaacaaac gcttttattg tgtaattttt gtgacgtata tcaggttttt 240
aagcaccctg tggcgctcat actggcaacc tgttgatatt aagcaacact cttcactcac 300
ggaattaaca cgcacagtaa aggtatacgc atg aaa aaa cgt gtt ctg gca gtt  354
                                Met Lys Lys Arg Val Leu Ala Val
                                  1               5
tgt ttt gcc gca ttg ttc tct tct cag gcc ctg gcg ctg gtc gct acc   402
Cys Phe Ala Ala Leu Phe Ser Ser Gln Ala Leu Ala Leu Val Ala Thr
     10                  15                  20
ggc aac gac act acc acg aaa ccg gat ctc tac tac ctc aag aac agt   450
Gly Asn Asp Thr Thr Thr Lys Pro Asp Leu Tyr Tyr Leu Lys Asn Ser
 25                  30                  35                  40
```

```
gaa gcc att aac agc ctg gcg ctg ttg ccg cca cca ccg gcg gtg ggc   498
Glu Ala Ile Asn Ser Leu Ala Leu Leu Pro Pro Pro Pro Ala Val Gly
                45                  50                  55
tcc att gcg ttt ctc aac gat cag gcc atg tat gaa cag ggg cgc ctg   546
Ser Ile Ala Phe Leu Asn Asp Gln Ala Met Tyr Glu Gln Gly Arg Leu
            60                  65                  70
ctg cgc aac acc gaa cgc ggt aag ctg gcg gcg gaa gat gca aac ctg   594
Leu Arg Asn Thr Glu Arg Gly Lys Leu Ala Ala Glu Asp Ala Asn Leu
        75                  80                  85
agc agt ggc ggg gtg gcg aat gct ttc tcc ggc gcg ttt ggt agc ccg   642
Ser Ser Gly Gly Val Ala Asn Ala Phe Ser Gly Ala Phe Gly Ser Pro
    90                  95                  100
atc acc gaa aaa gac gcc ccg gcg ctg cat aaa tta ctg acc aat atg   690
Ile Thr Glu Lys Asp Ala Pro Ala Leu His Lys Leu Leu Thr Asn Met
105                 110                 115                 120
att gag gac gcc ggg gat ctg gcg acc cgc agc gcg aaa gat cac tat   738
Ile Glu Asp Ala Gly Asp Leu Ala Thr Arg Ser Ala Lys Asp His Tyr
                125                 130                 135
atg cgc att cgt ccg ttc gcg ttt tat ggg gtc tct acc tgt aat acc   786
Met Arg Ile Arg Pro Phe Ala Phe Tyr Gly Val Ser Thr Cys Asn Thr
            140                 145                 150
acc gag cag gac aaa ctg tcc aaa aat ggc tct tat ccg tcc ggg cat   834
Thr Glu Gln Asp Lys Leu Ser Lys Asn Gly Ser Tyr Pro Ser Gly His
        155                 160                 165
acc tct atc ggc tgg gct act gcg ctg gtg ctg gca gag atc aac cct   882
Thr Ser Ile Gly Trp Ala Thr Ala Leu Val Leu Ala Glu Ile Asn Pro
    170                 175                 180
cag cgc cag aac gag atc ctg aaa cgc ggt tat gag ctg ggc cag agc   930
Gln Arg Gln Asn Glu Ile Leu Lys Arg Gly Tyr Glu Leu Gly Gln Ser
185                 190                 195                 200
cgg gtg att tgc ggc tac cac tgg cag agt gat gtg gat gcc gcg cgg   978
Arg Val Ile Cys Gly Tyr His Trp Gln Ser Asp Val Asp Ala Ala Arg
                205                 210                 215
gta gtg gga tct gcc gtt gtg gcg acc ctg cat acc aac ccg gcg ttc   1026
Val Val Gly Ser Ala Val Val Ala Thr Leu His Thr Asn Pro Ala Phe
            220                 225                 230
cag cag cag ttg cag aaa gcg aag gcc gaa ttc gcc cag cat cag aag   1074
Gln Gln Gln Leu Gln Lys Ala Lys Ala Glu Phe Ala Gln His Gln Lys
        235                 240                 245
aaa taatcctgac taccgccttg ccttgcaggg cggtagtggt ttccactggc        1127
Lys
cccgattcgc tattcccaca gtaataatga cggtatatga ttttgtgcaa cgaaaaggtt 1187
gtgtcacgcc acagcttata agatcatgtg ccgttaac                         1225
```

【００６７】

<;210>; 2
<;211>; 249
<;212>; PRT
<;213>; Escherichia blattae
<;400>; 2

```
Met Lys Lys Arg Val Leu Ala Val Cys Phe Ala Ala Leu Phe Ser Ser
```

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Gln Ala Leu Ala Leu Val Ala Thr Gly Asn Asp Thr Thr Thr Lys Pro
            20                  25                  30
Asp Leu Tyr Tyr Leu Lys Asn Ser Glu Ala Ile Asn Ser Leu Ala Leu
        35                  40                  45
Leu Pro Pro Pro Pro Ala Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
    50                  55                  60
Ala Met Tyr Glu Gln Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys
65                  70                  75                  80
Leu Ala Ala Glu Asp Ala Asn Leu Ser Ser Gly Gly Val Ala Asn Ala
                85                  90                  95
Phe Ser Gly Ala Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Ala
            100                 105                 110
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125
Thr Arg Ser Ala Lys Asp His Tyr Met Arg Ile Arg Pro Phe Ala Phe
    130                 135                 140
Tyr Gly Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys
145                 150                 155                 160
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                165                 170                 175
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
            180                 185                 190
Arg Gly Tyr Glu Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205
Gln Ser Asp Val Asp Ala Ala Arg Val Val Gly Ser Ala Val Val Ala
    210                 215                 220
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
225                 230                 235                 240
Ala Glu Phe Ala Gln His Gln Lys Lys
                245
```

【００６８】

```
<;210>; 3
<;211>; 1344
<;212>; DNA
<;213>; Morganella morganii
<;220>;
<;221>; CDS
<;222>; (316)..(1062)
<;400>; 3
gaattccgga aaatttcatt cattttaatt gttaagaata tgctggcaaa aacaaaaccc 60
aatgctttat attttcttat aatatctgtg tgttatcttt ttcaatacta tcggtcaggt 120
cttatttatc cgttcgttaa caaaagccat gctgtttctg tcaaattatc tgaaaatcat 180
catcaaaaat acttacctgt cttccgtctg tttcgtcaca ctttttttgaa agagttaaca 240
tcaatttgca tctctccgcc ctacactggc agacaggttt ctgagtaata ctgttgtatc 300
tgataaggag atgtc atg aag aag aat att atc gcc ggt tgt ctg ttc tca  351
                Met Lys Lys Asn Ile Ile Ala Gly Cys Leu Phe Ser
                  1               5                  10
ctg ttt tcc ctt tcc gcg ctg gcc gcg atc ccg gcg ggc aac gat gcc   399
Leu Phe Ser Leu Ser Ala Leu Ala Ala Ile Pro Ala Gly Asn Asp Ala
```

```
         15                  20                  25
acc acc aag ccg gat tta tat tat ctg aaa aat gaa cag gct atc gac   447
Thr Thr Lys Pro Asp Leu Tyr Tyr Leu Lys Asn Glu Gln Ala Ile Asp
     30                  35                  40
agc ctg aaa ctg tta ccg cca ccg ccg gaa gtc ggc agt att cag ttt   495
Ser Leu Lys Leu Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Gln Phe
 45                  50                  55                  60
tta aat gat cag gca atg tat gag aaa ggc cgt atg ctg cgc aat acc   543
Leu Asn Asp Gln Ala Met Tyr Glu Lys Gly Arg Met Leu Arg Asn Thr
                 65                  70                  75
gag cgc gga aaa cag gca cag gca gat gct gac ctg gcc gca ggg ggt   591
Glu Arg Gly Lys Gln Ala Gln Ala Asp Ala Asp Leu Ala Ala Gly Gly
             80                  85                  90
gtg gca acc gca ttt tca ggg gca ttc ggc tat ccg ata acc gaa aaa   639
Val Ala Thr Ala Phe Ser Gly Ala Phe Gly Tyr Pro Ile Thr Glu Lys
         95                 100                 105
gac tct ccg gag ctg tat aaa ctg ctg acc aat atg att gag gat gcc   687
Asp Ser Pro Glu Leu Tyr Lys Leu Leu Thr Asn Met Ile Glu Asp Ala
    110                 115                 120
ggt gat ctt gcc acc cgc tcc gcc aaa gaa cat tac atg cgc atc cgg   735
Gly Asp Leu Ala Thr Arg Ser Ala Lys Glu His Tyr Met Arg Ile Arg
125                 130                 135                 140
ccg ttt gcg ttt tac ggc aca gaa acc tgt aat acc aaa gat cag aaa   783
Pro Phe Ala Phe Tyr Gly Thr Glu Thr Cys Asn Thr Lys Asp Gln Lys
                145                 150                 155
aaa ctc tcc acc aac gga tct tac ccg tca ggt cat acg tct atc ggc   831
Lys Leu Ser Thr Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly
            160                 165                 170
tgg gca acc gca ctg gtg ctg gcg gaa gtg aac ccg gca aat cag gat   879
Trp Ala Thr Ala Leu Val Leu Ala Glu Val Asn Pro Ala Asn Gln Asp
        175                 180                 185
gcg att ctg gaa cgg ggt tat cag ctc gga cag agc cgg gtg att tgc   927
Ala Ile Leu Glu Arg Gly Tyr Gln Leu Gly Gln Ser Arg Val Ile Cys
    190                 195                 200
ggc tat cac tgg cag agt gat gtg gat gcc gcg cgg att gtc ggt tca   975
Gly Tyr His Trp Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser
205                 210                 215                 220
gcc gct gtc gcg aca tta cat tcc gat ccg gca ttt cag gcg cag tta  1023
Ala Ala Val Ala Thr Leu His Ser Asp Pro Ala Phe Gln Ala Gln Leu
                225                 230                 235
gcg aaa gcc aaa cag gaa ttt gca caa aaa tca cag aaa taaaagcagt   1072
Ala Lys Ala Lys Gln Glu Phe Ala Gln Lys Ser Gln Lys
            240                 245
gatatctggt cagggcagtg caatatctgc cctgaaatcc ctgtttattc ccacatccag 1132
cggtcttccc gatcccagcc ttttgttttc atgcagctgt agaaatagcg gttgcggctg 1192
tcttcattca catccatcac ataactttcc gttaccggtg tctgctcttt gtaggttttg 1252
ctgttaccgc agtcatcgtc ttttttgcag cgtttctcca catcccgcat cacactgcgc 1312
tgagcaactt cattttcac cggataaagc tt                               1344
```

【００６９】

<;210>; 4

<;211>; 249
<;212>; PRT
<;213>; Morganella morganii
<;400>; 4

```
Met Lys Lys Asn Ile Ile Ala Gly Cys Leu Phe Ser Leu Phe Ser Leu
  1               5                  10                  15
Ser Ala Leu Ala Ala Ile Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
            20                  25                  30
Asp Leu Tyr Tyr Leu Lys Asn Glu Gln Ala Ile Asp Ser Leu Lys Leu
        35                  40                  45
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Gln Phe Leu Asn Asp Gln
    50                  55                  60
Ala Met Tyr Glu Lys Gly Arg Met Leu Arg Asn Thr Glu Arg Gly Lys
65                  70                  75                  80
Gln Ala Gln Ala Asp Ala Asp Leu Ala Ala Gly Gly Val Ala Thr Ala
                85                  90                  95
Phe Ser Gly Ala Phe Gly Tyr Pro Ile Thr Glu Lys Asp Ser Pro Glu
            100                 105                 110
Leu Tyr Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125
Thr Arg Ser Ala Lys Glu His Tyr Met Arg Ile Arg Pro Phe Ala Phe
    130                 135                 140
Tyr Gly Thr Glu Thr Cys Asn Thr Lys Asp Gln Lys Lys Leu Ser Thr
145                 150                 155                 160
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                165                 170                 175
Leu Val Leu Ala Glu Val Asn Pro Ala Asn Gln Asp Ala Ile Leu Glu
            180                 185                 190
Arg Gly Tyr Gln Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Ala Val Ala
    210                 215                 220
Thr Leu His Ser Asp Pro Ala Phe Gln Ala Gln Leu Ala Lys Ala Lys
225                 230                 235                 240
Gln Glu Phe Ala Gln Lys Ser Gln Lys
                245
```

【００７０】

<;210>; 5
<;211>; 991
<;212>; DNA
<;213>; Salmonella typhimurium
<;220>;
<;221>; CDS
<;222>; (132)..(827)
<;400>; 5

```
cagtccggta tggacagacg ataatgccag gcgcagcgtc ctgctttttt acctgtatgt 60
tgaataacca ttgcaataaa tcattatagg attacatctg tttattattg cctgatccgg 120
agtgagtctt t atg aaa agt cgt tat tta gta ttt ttt cta cca ctg atc  170
            Met Lys Ser Arg Tyr Leu Val Phe Phe Leu Pro Leu Ile
              1               5                  10
```

```
gta gct aaa tat aca tca gca gaa aca gtg caa ccc ttt cat tct cct   218
Val Ala Lys Tyr Thr Ser Ala Glu Thr Val Gln Pro Phe His Ser Pro
     15                  20                  25
gaa gaa tca gtg aac agt cag ttc tac tta cca cca ccg cca ggt aat   266
Glu Glu Ser Val Asn Ser Gln Phe Tyr Leu Pro Pro Pro Pro Gly Asn
 30                  35                  40                  45
gat gat ccg gct tac cgc tat gat aag gag gct tat ttt aag ggc tat   314
Asp Asp Pro Ala Tyr Arg Tyr Asp Lys Glu Ala Tyr Phe Lys Gly Tyr
                 50                  55                  60
gcg ata aag ggt tcc ccg cga tgg aaa caa gct gct gag gat gca gat   362
Ala Ile Lys Gly Ser Pro Arg Trp Lys Gln Ala Ala Glu Asp Ala Asp
            65                  70                  75
gta agc gtg gaa aat ata gcc aga ata ttc tcg cca gta gtg ggt gct   410
Val Ser Val Glu Asn Ile Ala Arg Ile Phe Ser Pro Val Val Gly Ala
        80                  85                  90
aaa att aac ccc aaa gat acg cca gaa acc tgg aat atg tta aag aat   458
Lys Ile Asn Pro Lys Asp Thr Pro Glu Thr Trp Asn Met Leu Lys Asn
     95                 100                 105
ctt ctg aca atg ggc ggc tac tac gct act gct tcg gca aaa aaa tat   506
Leu Leu Thr Met Gly Gly Tyr Tyr Ala Thr Ala Ser Ala Lys Lys Tyr
110                 115                 120                 125
tat atg cgt acc cgc ccc ttt gtc tta ttt aat cat tcc acc tgc cgt   554
Tyr Met Arg Thr Arg Pro Phe Val Leu Phe Asn His Ser Thr Cys Arg
                130                 135                 140
cct gaa gat gag aat act ttg cga aaa aat ggc tct tac cct tcc ggg   602
Pro Glu Asp Glu Asn Thr Leu Arg Lys Asn Gly Ser Tyr Pro Ser Gly
            145                 150                 155
cat act gct tat ggt aca ctt ctg gca tta gta tta tcc gag gcc aga   650
His Thr Ala Tyr Gly Thr Leu Leu Ala Leu Val Leu Ser Glu Ala Arg
        160                 165                 170
ccg gaa cgc gcg cag gag ctc gcc aga cgc gga tgg gag ttc ggg caa   698
Pro Glu Arg Ala Gln Glu Leu Ala Arg Arg Gly Trp Glu Phe Gly Gln
    175                 180                 185
agc aga gtg ata tgc ggt gct cac tgg caa agc gat gtt gat gct ggc   746
Ser Arg Val Ile Cys Gly Ala His Trp Gln Ser Asp Val Asp Ala Gly
190                 195                 200                 205
cgt tat gtg gga gca gta gag ttt gca aga ctg caa aca atc ccg gct   794
Arg Tyr Val Gly Ala Val Glu Phe Ala Arg Leu Gln Thr Ile Pro Ala
                210                 215                 220
ttt cag aag tca ctg gca aaa tcc gtg agg agc tgaacgacaa aaataattta 847
Phe Gln Lys Ser Leu Ala Lys Ser Val Arg Ser
            225                 230
ttgagtaaag aagatcaccc caaacttaat tactgaaggt gaaagtcttc ccgcaaactg 907
gccacagcaa atgaaaggaa gtgcaactgc gtaggggcgg ccgggcgtgg agaatgcctt 967
tggtttcccc gattcgcatg aatt                                       991
```

【００７１】

<;210>; 6
<;211>; 232
<;212>; PRT
<;213>; Salmonella typhimurium

<;400>; 6
Met Lys Ser Arg Tyr Leu Val Phe Phe Leu Pro Leu Ile Val Ala Lys
1 5 10 15
Tyr Thr Ser Ala Glu Thr Val Gln Pro Phe His Ser Pro Glu Glu Ser
20 25 30
Val Asn Ser Gln Phe Tyr Leu Pro Pro Pro Pro Gly Asn Asp Asp Pro
35 40 45
Ala Tyr Arg Tyr Asp Lys Glu Ala Tyr Phe Lys Gly Tyr Ala Ile Lys
50 55 60
Gly Ser Pro Arg Trp Lys Gln Ala Ala Glu Asp Ala Asp Val Ser Val
65 70 75 80
Glu Asn Ile Ala Arg Ile Phe Ser Pro Val Val Gly Ala Lys Ile Asn
85 90 95
Pro Lys Asp Thr Pro Glu Thr Trp Asn Met Leu Lys Asn Leu Leu Thr
100 105 110
Met Gly Gly Tyr Tyr Ala Thr Ala Ser Ala Lys Lys Tyr Tyr Met Arg
115 120 125
Thr Arg Pro Phe Val Leu Phe Asn His Ser Thr Cys Arg Pro Glu Asp
130 135 140
Glu Asn Thr Leu Arg Lys Asn Gly Ser Tyr Pro Ser Gly His Thr Ala
145 150 155 160
Tyr Gly Thr Leu Leu Ala Leu Val Leu Ser Glu Ala Arg Pro Glu Arg
165 170 175
Ala Gln Glu Leu Ala Arg Arg Gly Trp Glu Phe Gly Gln Ser Arg Val
180 185 190
Ile Cys Gly Ala His Trp Gln Ser Asp Val Asp Ala Gly Arg Tyr Val
195 200 205
Gly Ala Val Glu Phe Ala Arg Leu Gln Thr Ile Pro Ala Phe Gln Lys
210 215 220
Ser Leu Ala Lys Ser Val Arg Ser
225 230

【００７２】

<;210>; 7
<;211>; 1335
<;212>; DNA
<;213>; Zymomonas mobilis
<;220>;
<;221>; CDS
<;222>; (317)..(1108)
<;400>; 7
gtgccttata tcacgggggg atcagtctgc ggcaattggt ggcatggcac gcgctacggc 60
acgccggaag gcttcatggt cgtcaaagtc gaaaagggta aagtcattcc gcattacgaa 120
agctatggct tccacacgat agacccgcgc aacacataat tgtcttatta tagccacatg 180
atatttttat attacaattt taaactaaaa ttaagaatta aattcttgaa ataaaggttt 240
ttttattaaa aggataggaa atgtcgtgaa atcggcattt tctatccata ttatataaca 300
agggaagact gacgac atg ata aaa gtc ccg cgg ttc atc tgt atg atc gcg 352
Met Ile Lys Val Pro Arg Phe Ile Cys Met Ile Ala
1 5 10
ctt aca tcc ggc gtt ctg gca agc ggc ctt tct caa agc gtt tca gct 400
Leu Thr Ser Gly Val Leu Ala Ser Gly Leu Ser Gln Ser Val Ser Ala

```
         15                  20                  25
cat aca gaa aaa agt gaa ccc tcc tcg act tat cat ttc cac agc gat   448
His Thr Glu Lys Ser Glu Pro Ser Ser Thr Tyr His Phe His Ser Asp
     30                  35                  40
ccc ctt ctt tac ctt gcg ccc cca ccc act tcc ggc agt cca tta cag   496
Pro Leu Leu Tyr Leu Ala Pro Pro Pro Thr Ser Gly Ser Pro Leu Gln
 45                  50                  55                  60
gcg cat gat gat caa acc ttt aac agc acc aga caa tta aaa ggt agc   544
Ala His Asp Asp Gln Thr Phe Asn Ser Thr Arg Gln Leu Lys Gly Ser
                 65                  70                  75
acg cgc tgg gca ttg gca act caa gat gcc gat ctt cat ctc gct tca   592
Thr Arg Trp Ala Leu Ala Thr Gln Asp Ala Asp Leu His Leu Ala Ser
             80                  85                  90
gtt ctc aaa gac tat gcc tgc gcc gca gga atg aat ctc gat att gcg   640
Val Leu Lys Asp Tyr Ala Cys Ala Ala Gly Met Asn Leu Asp Ile Ala
         95                 100                 105
caa tta ccg cat ctt gcc aat ttg att aaa cgc gca ctt cgc acc gaa   688
Gln Leu Pro His Leu Ala Asn Leu Ile Lys Arg Ala Leu Arg Thr Glu
    110                 115                 120
tat gac gat att ggc aga gcc aaa aat aac tgg aat cgc aaa cga cct   736
Tyr Asp Asp Ile Gly Arg Ala Lys Asn Asn Trp Asn Arg Lys Arg Pro
125                 130                 135                 140
ttt gtg gat acc gat caa ccc atc tgc acg gaa aaa gat cgc gaa ggt   784
Phe Val Asp Thr Asp Gln Pro Ile Cys Thr Glu Lys Asp Arg Glu Gly
                145                 150                 155
ctg gga aaa caa ggc tcc tat cct tca ggt cat acg act atc ggt tgg   832
Leu Gly Lys Gln Gly Ser Tyr Pro Ser Gly His Thr Thr Ile Gly Trp
            160                 165                 170
agc gtt gcg ctc att ctg gct gaa ttg atc ccc gat cat gcg gcg aat   880
Ser Val Ala Leu Ile Leu Ala Glu Leu Ile Pro Asp His Ala Ala Asn
        175                 180                 185
att ttg cag cgt ggc caa att ttt gga acc agc cgg att gtc tgc ggc   928
Ile Leu Gln Arg Gly Gln Ile Phe Gly Thr Ser Arg Ile Val Cys Gly
    190                 195                 200
gcc cat tgg ttc agc gat gtg cag gca ggc tat atc atg gca tcg ggc   976
Ala His Trp Phe Ser Asp Val Gln Ala Gly Tyr Ile Met Ala Ser Gly
205                 210                 215                 220
gaa att gca gct tta cat ggg gat gcc gat ttc cgc cga gat atg gaa   1024
Glu Ile Ala Ala Leu His Gly Asp Ala Asp Phe Arg Arg Asp Met Glu
                225                 230                 235
tta gct cgg aaa gaa tta gaa aag gca cgc aca tca gcg cac acg cca   1072
Leu Ala Arg Lys Glu Leu Glu Lys Ala Arg Thr Ser Ala His Thr Pro
            240                 245                 250
gac gat ctt cta tgc aag att gaa caa agc gct cgc taaattcaat        1118
Asp Asp Leu Leu Cys Lys Ile Glu Gln Ser Ala Arg
        255                 260
caagtattat ttcaacaagg ggaaagattg cttgctgtaa tttttggata tcaaacaggc 1178
gaaaaaatga aagagcgcac gctctttcaa aggcaattcg atttagtccg gtggcattct 1238
cacgccacaa accaaatcat aaataaccgc ctcttttccg ccagataact gcaaaattat 1298
agaataccga cagctggaat atcgtcactt ttcctag                          1335
```

【００７３】

<;210>; 8
<;211>; 264
<;212>; PRT
<;213>; Zymomonas mobilis
<;400>; 8

```
Met Ile Lys Val Pro Arg Phe Ile Cys Met Ile Ala Leu Thr Ser Gly
  1               5                  10                  15
Val Leu Ala Ser Gly Leu Ser Gln Ser Val Ser Ala His Thr Glu Lys
             20                  25                  30
Ser Glu Pro Ser Ser Thr Tyr His Phe His Ser Asp Pro Leu Leu Tyr
         35                  40                  45
Leu Ala Pro Pro Pro Thr Ser Gly Ser Pro Leu Gln Ala His Asp Asp
     50                  55                  60
Gln Thr Phe Asn Ser Thr Arg Gln Leu Lys Gly Ser Thr Arg Trp Ala
 65                  70                  75                  80
Leu Ala Thr Gln Asp Ala Asp Leu His Leu Ala Ser Val Leu Lys Asp
                 85                  90                  95
Tyr Ala Cys Ala Ala Gly Met Asn Leu Asp Ile Ala Gln Leu Pro His
            100                 105                 110
Leu Ala Asn Leu Ile Lys Arg Ala Leu Arg Thr Glu Tyr Asp Asp Ile
        115                 120                 125
Gly Arg Ala Lys Asn Asn Trp Asn Arg Lys Arg Pro Phe Val Asp Thr
    130                 135                 140
Asp Gln Pro Ile Cys Thr Glu Lys Asp Arg Glu Gly Leu Gly Lys Gln
145                 150                 155                 160
Gly Ser Tyr Pro Ser Gly His Thr Thr Ile Gly Trp Ser Val Ala Leu
                165                 170                 175
Ile Leu Ala Glu Leu Ile Pro Asp His Ala Ala Asn Ile Leu Gln Arg
            180                 185                 190
Gly Gln Ile Phe Gly Thr Ser Arg Ile Val Cys Gly Ala His Trp Phe
        195                 200                 205
Ser Asp Val Gln Ala Gly Tyr Ile Met Ala Ser Gly Glu Ile Ala Ala
    210                 215                 220
Leu His Gly Asp Ala Asp Phe Arg Arg Asp Met Glu Leu Ala Arg Lys
225                 230                 235                 240
Glu Leu Glu Lys Ala Arg Thr Ser Ala His Thr Pro Asp Asp Leu Leu
                245                 250                 255
Cys Lys Ile Glu Gln Ser Ala Arg
            260
```

【００７４】

<;210>; 9
<;211>; 1650
<;212>; DNA
<;213>; Enterobacter aerogenes
<;220>;
<;221>; CDS
<;222>; (344)..(1087)
<;220>;
<;221>; mat#peptide

<;222>; (404)..(1087)
<;220>;
<;221>; sig#peptide
<;222>; (344)..(403)
<;400>; 9

```
gtcgacaaac ttcgcctgct cgctatgcag aatggtttcc agcactttag gggaaatttt 60
acaaccgcaa ccggctccgt ggctgtattg cgttaaacga atagcttgct cgctcatgga 120
catctcctgt cattgcaatc ccgctatggt agcgcccaaa cggcaaggtg ataagtgcga 180
cagtccgaaa tcgcgagtgg ttgctcatta agcagacaaa tatgcgtttt tgcgataccg 240
aacaattttt tcaatgtgat tttaactttt acttacagat gacaaaaatg tgactaaaaa 300
caaaaccatt gttctggaca tataacaccg taaggaaatg tag atg aaa aag cgc   355
                                              Met Lys Lys Arg
                                              -20
gtt ctc gcc ctc tgc ctc gcc agc ctg ttt tcc gtt aac gct ttc gcg   403
Val Leu Ala Leu Cys Leu Ala Ser Leu Phe Ser Val Asn Ala Phe Ala
    -15                 -10                 -5                 -1
ctg gtc cct gcc ggc aat gat gca acc acc aaa ccg gat ctc tat tat   451
Leu Val Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro Asp Leu Tyr Tyr
  1               5                  10                  15
ctg aaa aat gca cag gcc atc gat agt ctg gcg ctg ttg ccg ccg ccg   499
Leu Lys Asn Ala Gln Ala Ile Asp Ser Leu Ala Leu Leu Pro Pro Pro
            20                  25                  30
ccg gaa gtt ggc agc atc gca ttt tta aac gat cag gcg atg tat gag   547
Pro Glu Val Gly Ser Ile Ala Phe Leu Asn Asp Gln Ala Met Tyr Glu
        35                  40                  45
aaa gga cgg ctg ttg cgc aat acc gaa cgt ggc aag cag gcg cag gca   595
Lys Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys Gln Ala Gln Ala
    50                  55                  60
gat gct gac ctg gcc gcc ggc gac gtc gcg aat gcc ttc tcc agc gct   643
Asp Ala Asp Leu Ala Ala Gly Asp Val Ala Asn Ala Phe Ser Ser Ala
65                  70                  75                  80
ttt ggt tcg ccc atc acc gaa aaa gac gcg ccg cag tta cat aag ctg   691
Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Gln Leu His Lys Leu
                85                  90                  95
ctg aca aat atg att gag gat gcc ggc gat ctg gcc acc cgc agc gcg   739
Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala Thr Arg Ser Ala
            100                 105                 110
aaa gag aaa tat atg cgc att cgc ccg ttt gcg ttc tac ggc gtt tca   787
Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe Tyr Gly Val Ser
        115                 120                 125
acc tgt aac act aaa gac cag gac aag ctg tcg aaa aac gga tct tac   835
Thr Cys Asn Thr Lys Asp Gln Asp Lys Leu Ser Lys Asn Gly Ser Tyr
    130                 135                 140
cct tcc ggc cat acc tct acc ggt tgg gca acc gcg ctg gta ctg gcg   883
Pro Ser Gly His Thr Ser Thr Gly Trp Ala Thr Ala Leu Val Leu Ala
145                 150                 155                 160
gag atc aat ccg cag cgg caa aac gaa att ctc aaa cgc ggc tat gaa   931
Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys Arg Gly Tyr Glu
                165                 170                 175
ttg ggc gaa agc cgg gtt atc tgc ggc tat cat tgg cag agc gat gtc   979
```

```
Leu Gly Glu Ser Arg Val Ile Cys Gly Tyr His Trp Gln Ser Asp Val
            180                 185                 190
gat gcg gcg cgg ata gtc ggc tcg gcg gtg gtg gcg acc ctg cat acc   1027
Asp Ala Ala Arg Ile Val Gly Ser Ala Val Val Ala Thr Leu His Thr
        195                 200                 205
aac ccg gcc ttc caa cag cag ttg cag aaa gca aag gat gaa ttc gcc   1075
Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys Asp Glu Phe Ala
    210                 215                 220
aaa acg cag aag taacgtcatc gccgttgaac tcccggaggc ggcgcttaac       1127
Lys Thr Gln Lys
225
gcgccttctc cgggctacta aatcgcacag cgctgtagcc ccggtaagcg ccagcgccac 1187
cggggatttt gagatagcca gcaccagtag tttcagccag cgtgatgaat acattaacgg 1247
caggccgcat gagtcgtaga tactgttatc ggtttgcaac ttttttaagg ttttttcccg 1307
gaggcggcgc gctgcgcctt ctccgggcta ctaaatcgca cagcgctgta gccccggtaa 1367
gcggcagcgc caccgggggt aacaagcgca gattcagaag cgcgtgacga acggcgcggt 1427
atccgggcgc gtaaacatgg ttgatgcttt taactgcggc gtgccaaggt agaggaaacc 1487
gacaattttg tcctgttcgc ggcagccaaa gccttcgcgg acaaccggac tctcggttaa 1547
cgcaccgata cgccagatac cgttatagcc ctgcgccact gcggccattt gcatcgccat 1607
caccgcacat cccgcggaca tctcctgttc ccacagcggt acc                   1650
```

【００７５】

<;210>; 10
<;211>; 248
<;212>; PRT
<;213>; Enterobacter aerogenes
<;400>; 10

```
Met Lys Lys Arg Val Leu Ala Leu Cys Leu Ala Ser Leu Phe Ser Val
-20                 -15                 -10                  -5
Asn Ala Phe Ala Leu Val Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
            -1   1                   5                  10
Asp Leu Tyr Tyr Leu Lys Asn Ala Gln Ala Ile Asp Ser Leu Ala Leu
         15                  20                  25
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
     30                  35                  40
Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys
 45                  50                  55                  60
Gln Ala Gln Ala Asp Ala Asp Leu Ala Ala Gly Asp Val Ala Asn Ala
                 65                  70                  75
Phe Ser Ser Ala Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Gln
             80                  85                  90
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
         95                  100                 105
Thr Arg Ser Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe
    110                 115                 120
Tyr Gly Val Ser Thr Cys Asn Thr Lys Asp Gln Asp Lys Leu Ser Lys
125                 130                 135                 140
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Thr Gly Trp Ala Thr Ala
                145                 150                 155
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
            160                 165                 170
```

Arg Gly Tyr Glu Leu Gly Glu Ser Arg Val Ile Cys Gly Tyr His Trp
175 180 185
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Val Val Ala
190 195 200
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
205 210 215 220
Asp Glu Phe Ala Lys Thr Gln Lys
225

【００７６】

<;210>; 11
<;211>; 25
<;212>; DNA
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acid sequence around mutation
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【００７９】

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【００８０】

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Asn Leu Ser Tyr Gly Asp Val
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【００８２】

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【００８５】

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【００８６】

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acid sequence around mutation
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【００８９】

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【００９０】

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acid sequence around mutation
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【００９２】

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【００９３】

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acid sequence around mutation
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Asn Leu Ser Glu Gly Asp Val
1 5

【００９４】

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【００９５】

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acid sequence around mutation
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【００９７】

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【００９８】

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【００９９】

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acid sequence around mutation
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Asn Leu Ser Thr Gly Asp Val
1 5

【０１００】

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【０１０１】

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【０１０２】

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1 5

【０１０３】

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【０１０４】

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【０１０５】

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1 5

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【０１０７】

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【０１０８】

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Asn Leu Ser Gln Gly Asp Val
1 5

【０１０９】

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【０１１０】

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【０１１１】

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Asn Leu Ser Lys Gly Asp Val
1 5

【０１１２】

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【０１１３】

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【０１１４】

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1 5

【０１１５】

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【０１１６】

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gtttggactc gcgcccgcta caccg 25

【０１１７】

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Asn Leu Ser Ala Gly Asp Val
1 5

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【０１１９】

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【０１２０】

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1 5

【０１２１】

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【０１２２】

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【０１２３】

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Asn Leu Ser Gly Gly Asp Val
1 5

【０１２４】

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【０１２５】

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gtttggactc ggtgccgcta caccg 25

【０１２６】

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Asn Leu Ser His Gly Asp Val
1 5

【０１２７】

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cgaaaccgga ttggtactac ctcaa 25

【０１２８】

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gctttggcct aaccatgatg gagtt 25

【０１２９】

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Lys Pro Asp Trp Tyr Tyr Leu
1 5

【０１３０】

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atgcaaacct gtggagtggc gatgt 25

【０１３１】

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tacgtttgga cacctcaccg ctaca 25

【０１３２】

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Ala Asn Leu Trp Ser Gly Asp
1 5

【０１３３】

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acctgagcag ttgggatgtg gcgaa 25

【０１３４】

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tggactcgtc aaccctacac cgctt 25

【０１３５】

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Leu Ser Ser Trp Asp Val Ala
1 5

【０１３６】

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<;211>; 25

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<;400>; 71
ccaatatgat ttttgacgcc gggga 25

【０１３７】

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ggttatacta aaaactgcgg cccct 25

【０１３８】

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acid sequence around mutation
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Asn Met Ile Phe Asp Ala Gly
1 5

【０１３９】

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ccaatatgat ttgggacgcc gggga 25

【０１４０】

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ggttatacta aaccctgcgg cccct 25

【０１４１】

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acid sequence around mutation
<;400>; 76
Asn Met Ile Trp Asp Ala Gly
1 5

【０１４２】

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<;211>; 25
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cagacctggc ctttggcgat gtggc 25

【０１４３】

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<;400>; 78
gtctggaccg gaaaccgcta caccg 25

【０１４４】

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acid sequence around mutation
<;400>; 79
Asp Leu Ala Phe Gly Asp Val
1 5

【０１４５】

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cagacctggc cgaaggcgat gtggc 25

【０１４６】

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gtctggaccg gcttccgcta caccg 25

【０１４７】

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<;400>; 82
Asp Leu Ala Glu Gly Asp Val
1 5

【０１４８】

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<;213>; Artificial Sequence
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<;400>; 83
tgaccaatat ggacgaggac gccgg 25

【０１４９】

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<;211>; 25
<;212>; DNA
<;213>; Artificial Sequence
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<;223>; Description of Artificial Sequence:primer
<;400>; 84
actggttata cctgctcctg cggcc 25

【０１５０】

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<;223>; Description of Artificial Sequence: partial amino acid sequence around mutation
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Thr Asn Met Asp Glu Asp Ala
1 5

【０１５１】

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<;223>; Description of Artificial Sequence:primer
<;400>; 86
ggcatacctc taacggctgg gctac 25

【０１５２】

<;210>; 87
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ccgtatggag attgccgacc cgatg 25

【０１５３】

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<;223>; Description of Artificial Sequence: partial amino acid sequence around mutation
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His Thr Ser Asn Gly Trp Ala
1 5

【０１５４】

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<;400>; 89
accaggacaa attctccaaa aatgg 25

【０１５５】

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<;212>; DNA
<;213>; Artificial Sequence
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<;223>; Description of Artificial Sequence:primer
<;400>; 90
tggtcctgtt taagaggttt ttacc 25

【０１５６】

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<;211>; 7
<;212>; PRT
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<;223>; Description of Artificial Sequence: partial amino acid sequence around mutation
<;400>; 91

Gln Asp Lys Phe Ser Lys Asn
1 5

【０１５７】

<;210>; 92
<;211>; 25
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<;220>;
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<;400>; 92
accaggacaa aaaatccaaa aatgg 25

【０１５８】

<;210>; 93
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<;212>; DNA
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<;400>; 93
tggtcctgtt ttttaggttt ttacc 25

【０１５９】

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<;400>; 94
Gln Asp Lys Lys Ser Lys Asn
1 5

【０１６０】

<;210>; 95
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<;400>; 95
accaggacaa agaatccaaa aatgg 25

【０１６１】

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<;212>; DNA
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<;400>; 96

tggtcctgtt tcttaggttt ttacc 25

【０１６２】

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<;212>; PRT
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Gln Asp Lys Glu Ser Lys Asn
1 5

【０１６３】

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<;400>; 98
Leu Val Pro Ala Gly
5

【０１６４】

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cttacagatg actataatgt gactaaaaac 30

【０１６５】

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gtttttagtc acattatag tcatctgtaag 30

【０１６６】

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<;400>; 101
tctaccggtt gggcatccgc gctggtactg gcg 33

【０１６７】

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<;400>; 102
cgccagtacc agcgcggatg cccaaccggt aga 33
MUT300

【０１６８】
<;210>; 103
<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 103
tccggccata cctcttccgg ttgggcatcc gcg 33

【０１６９】
<;210>; 104
<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 104
cgcggatgcc caaccggaag aggtatggcc gga 33

【０１７０】
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<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 105
gatgctgacc tggccgttgg cgacgtcgcg aat 33

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<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 106
attcgcgacg tcgccaacgg ccaggtcagc atc 33

【０１７２】
<;210>; 107
<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
<;220>;

<;223>; Description of Artificial Sequence:primer
<;400>; 107
ctgacaaata tgattctgga tgccggcgat ctg 33

【０１７３】

<;210>; 108
<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 108
cagatcgccg gcatccagaa tcatatttgt cag 33

【０１７４】

<;210>; 109
<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 109
gatgctgacc tggccatgg gcgacgtcgcg aat 33

【０１７５】

<;210>; 110
<;211>; 33
<;212>; DNA
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<;223>; Description of Artificial Sequence:primer
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attcgcgacg tcgcccatg gccaggtcagc atc 33

【０１７６】

<;210>; 111
<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence

<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 111
ctgacaaata tgattcagg atgccggcgat ctg 33

【０１７７】

<;210>; 112
<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
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<;223>; Description of Artificial Sequence:primer
<;400>; 112
cagatcgccg gcatcctga atcatatttgt cag 33

【０１７８】

<;210>; 113
<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 113
tccggccata cctctgctgg ttgggcatcc gcg 33

【０１７９】

<;210>; 114
<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 114
cgcggatgcc caaccagcag aggtatggcc gga 33

【０１８０】

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<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 115
tccggccata cctctggcgg ttgggcatcc gcg 33

【０１８１】

<;210>; 116
<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 116
cgcggatgcc caaccgccag aggtatggcc gga 33

【０１８２】

<;210>; 117
<;211>; 33
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<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 117
gatgctgacc tggccgaagg cgacgtcgcg aat 33

【０１８３】

<;210>; 118
<;211>; 33
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<;213>; Artificial Sequence
<;220>;

<;223>; Description of Artificial Sequence:primer

<;400>; 118
attcgcgacg tcgccttcgg ccaggtcagc atc 33

【０１８４】

<;210>; 119
<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 119
gatgctgacc tggccaaagg cgacgtcgcg aat 33

【０１８５】

<;210>; 120
<;211>; 33
<;212>; DNA
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence:primer
<;400>; 120
attcgcgacg tcgcctttgg ccaggtcagc atc 33

【０１８６】

<;210>; 121
<;211>; 9
<;212>; PRT
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence: common motif
among acidic phosphatase family
<;220>;
<;221>; UNSURE
<;222>; (2,3,4,5,6,7)
<;223>; X=optional amino acid
<;400>; 121
Lys Xaa Xaa Xaa Xaa Xaa Xaa Arg Pro
1 5

【０１８７】

<;210>; 122
<;211>; 4
<;212>; PRT
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence: common motif
among acidic phosphatase family
<;400>; 122
Pro Ser Gly His
1

【０１８８】

<;210>; 123

<;211>; 12
<;212>; PRT
<;213>; Artificial Sequence
<;220>;
<;223>; Description of Artificial Sequence: common motif
among acidic phosphatase family
<;220>;
<;221>; UNSURE
<;222>; (3,4,5,6,7,9,10,11)
<;223>; X=optional amino acid
<;400>; 123
Ser Arg Xaa Xaa Xaa Xaa Xaa His Xaa Xaa Xaa Asp
1　　5　　10

【図面の簡単な説明】

【図１】ホスファターゼ活性あるいはリン酸基転移活性を有する活性部位の構成要素となる５つのアミノ酸残基と、これらの空間的位置関係をＣα原子間の距離として示した図である。

【図２】ＥＢ－ＡＰのアミノ酸配列を、モルガネラ・モルガニ、サルモネラ・チフィムリウム、ザイモモナス・モビリス由来酸性ホスファターゼのアミノ酸配列とアラインメントした図である。

【図３】ＥＢ－ＡＰ反応中間体アナログとイノシンの結合様式モデルの結晶構造を示すコンピューターグラフィックス（ＣＧ）の写真である。

【図４】ＥＢ－ＡＰの６量体分子の結晶構造を示すＣＧの写真である。

【図５】ＥＢ－ＡＰのサブユニットの結晶構造を示すＣＧの写真である。

【図６】ＥＢ－ＡＰの活性部位構造を示す図である。

【図７】部位特異的突然変異誘発法に使用したプライマーセットを示す図である。

【図８】部位特異的突然変異誘発法に使用したプライマーセットを示す図である。

【図９】ＥＢ－ＡＰとエンテロバクター・アエロゲネス由来酸性ホスファターゼ（ＥＡ－ＡＰ）のアミノ酸配列アラインメントをプログラムBLASTにより行った結果を示す図である。

【図１０】ＥＢ－ＡＰの構造の結晶学データ（１）を示す図である。

【図１１】ＥＢ－ＡＰの構造の結晶学データ（２）を示す図である。

【図１２】ＥＢ－ＡＰの構造の結晶学データ（３）を示す図である。

【図１３】ＥＢ－ＡＰの構造の結晶学データ（４）を示す図である。

【図１４】ＥＢ－ＡＰの構造の結晶学データ（５）を示す図である。

【図１５】ＥＢ－ＡＰの構造の結晶学データ（６）を示す図である。

【図１６】ＥＢ－ＡＰの構造の結晶学データ（７）を示す図である。

【図１７】ＥＢ－ＡＰの構造の結晶学データ（８）を示す図である。

【図１８】ＥＢ－ＡＰの構造の結晶学データ（９）を示す図である。

【図１９】ＥＢ－ＡＰの構造の結晶学データ（１０）を示す図である。

【図２０】ＥＢ－ＡＰの構造の結晶学データ（１１）を示す図である。

【図２１】ＥＢ－ＡＰの構造の結晶学データ（１２）を示す図である。

【図２２】ＥＢ－ＡＰの構造の結晶学データ（１３）を示す図である。

【図２３】ＥＢ－ＡＰの構造の結晶学データ（１４）を示す図である。

【図２４】ＥＢ－ＡＰの構造の結晶学データ（１５）を示す図である。

【図２５】ＥＢ－ＡＰの構造の結晶学データ（１６）を示す図である。

【図２６】ＥＢ－ＡＰの構造の結晶学データ（１７）を示す図である。

【図２７】ＥＢ－ＡＰの構造の結晶学データ（１８）を示す図である。

【図２８】ＥＢ－ＡＰの構造の結晶学データ（１９）を示す図である。

【図２９】ＥＢ－ＡＰの構造の結晶学データ（２０）を示す図である。

【図３０】ＥＢ－ＡＰの構造の結晶学データ（２１）を示す図である。

【図３１】ＥＢ－ＡＰの構造の結晶学データ（２２）を示す図である。

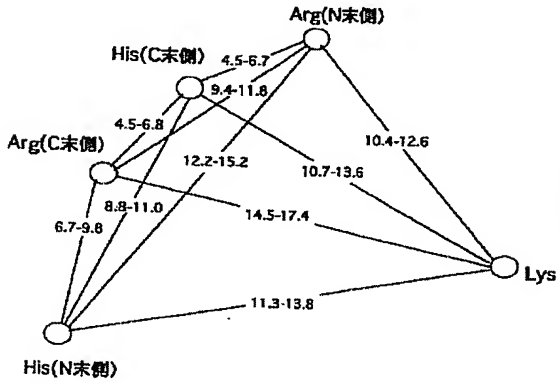
【図３２】ＥＢ－ＡＰの構造の結晶学データ（２３）を示す図である。

【図３３】ＥＢ－ＡＰの構造の結晶学データ（２４）を示す図である。

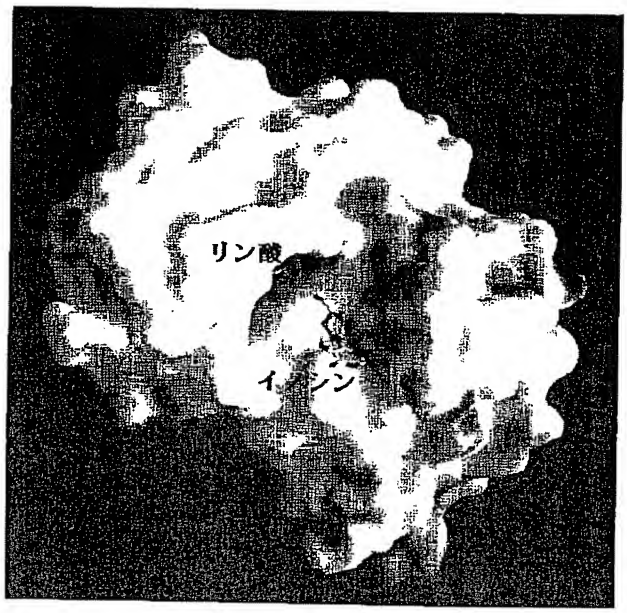
【図３４】ＥＢ－ＡＰの構造の結晶学データ（２５）を

示す図である。
【図３５】ＥＢ－ＡＰの構造の結晶学データ（２６）を示す図である。
【図３６】ＥＢ－ＡＰの構造の結晶学データ（２７）を示す図である。
【図３７】ＥＢ－ＡＰの構造の結晶学データ（２８）を示す図である。
【図３８】ＥＢ－ＡＰの構造の結晶学データ（２９）を示す図である。
【図３９】ＥＢ－ＡＰの構造の結晶学データ（３０）を示す図である。
【図４０】ＥＢ－ＡＰの構造の結晶学データ（３１）を示す図である。
【図４１】ＥＢ－ＡＰの構造の結晶学データ（３２）を示す図である。
【図４２】ＥＢ－ＡＰの構造の結晶学データ（３３）を示す図である。
【図４３】ＥＢ－ＡＰの構造の結晶学データ（３４）を示す図である。
【図４４】ＥＢ－ＡＰの構造の結晶学データ（３５）を示す図である。
【図４５】ＥＢ－ＡＰの構造の結晶学データ（３６）を示す図である。
【図４６】部位特異的突然変異誘発法に使用したプライマーセットを示す図である。
【図４７】部位特異的突然変異誘発法に使用したプライマーセットを示す図である。
【図４８】部位特異的突然変異誘発法に使用したプライマーセットを示す図である。

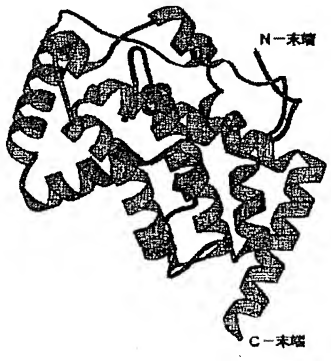
【図１】



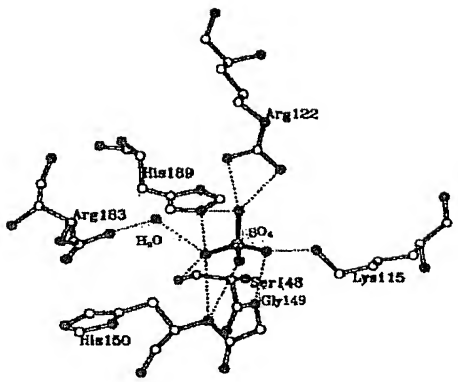
【図３】



【図５】



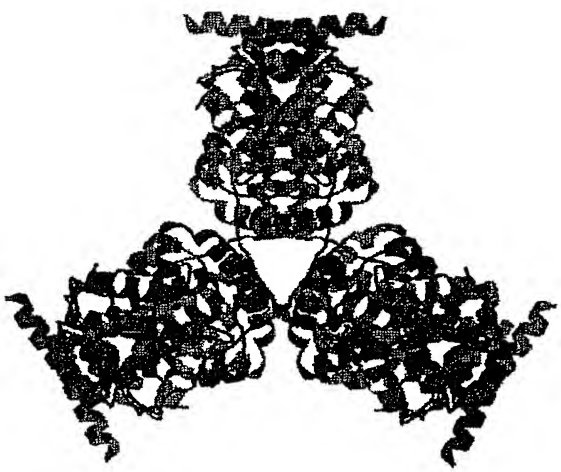
【図６】



【図２】

α1
α2
α3
α4
α5
α6
α7
α8
α9
α10
α11
α12
α13
E.blattae :LALVATGNDT TTKPDLYYLK NSEAINSLAL LPPPPAVGSI AFLNDQAMYE QGRLLRNTER GKLAAEDANL 70
M.morganii : AIPAGNDA TTKPDLYYLK NEQAIDSLKL LPPPPEVGSI QFLNDQAMYE KGRMLRNTER GKQAQADADL
S.typhimurium : KYT SAETVQPFHS PEESVNSQFY LPPPPGNDDP AYRYDKEAYF KGYAILGSPR WKQAAEDADV
Z.mobilis :ASGLSQSVSA HTEKSEPSST YHFHSDPLLY LAPPPTSGSP LQAHDDQTFN STRQLKGSTR WALATQDADL
E.blattae :SSGGVANAFS GAFGSPTTEK DAPALHKLLT NMIEDAGDLA TRSAKDHYMR IRPFAFYGVS TCNTTEQDKL 140
M.morganii :AAGGVATAFS GAFGYPTTEK DSPELVKLLT NMIEDAGDLA TRSAKEHYMR IRPFAFYGTE TCNTKDQKKL
S.typhimurium :SVENIARIFS PVVGAKINPK DTPETWNMLK NLLTMGGYYA TASAKKYYMR TRPFVLFNHS TCRPEDENTL
Z.mobilis :HLASVLKDYA CAAGMNLDIA QLPHLANLIK RALRTEYDDI GR-AKNNWNR KRPFVDTDQP ICTEKDREGL
E.blattae :SKNGSYPSGH TSIGWATALV LAEINPQRQN EILKRGYELG QSRVICGYHW QSRVDAARVV GSAVVATLET 210
M.morganii :STNGSYPSGH TSIGWATALV LAEVNPANQD AILERGYQLG QSRVICGYHW QSRVDAARIV GSAAVATLHS
S.typhimurium :RKNGSYPSGH TAYGTLLALV LSEARPERAQ ELARRGWEFG QSRVICGAHW QSRVDAGRYV GAVEFARLQT
Z.mobilis :GKQGSYPSGH TTIGWSVALI LAELIPDEAA NILQRGQIFG TSRIVCGAHW FSDVQAGYIM ASGEIAALHG
E.blattae :NPAFQQQLQK AKAEFAQHQK K
M.morganii :DPAFQAQLAK AKQEFAQKSQ K
S.typhimurium :IPAFQKSLAK VREELNDKNN LLSKEDHPKL NY
Z.mobilis :DADFRRDMEL ARKELEKART SAHTPDDLLC KIEQSAR

【図４】



【図７】

```
S72F(s)    5'-CA-AAC-CTG-AGC-TTT-GGC-GAT-GTG-GC-3'
S72F(as)   3'-GT-TTG-GAC-TCG-AAA-CCG-CTA-CAC-CG-5'
                N   L   S   F72  G   D   V
S72Y(s)    5'-CA-AAC-CTG-AGC-TAC-GGC-GAT-GTG-GC-3'
S72Y(as)   3'-GT-TTG-GAC-TCG-ATG-CCG-CTA-CAC-CG-5'
                N   L   S   Y72  G   D   V
S72W(s)    5'-CA-AAC-CTG-AGC-TGG-GGC-GAT-GTG-GC-3'
S72W(as)   3'-GT-TTG-GAC-TCG-ACC-CCG-CTA-CAC-CG-5'
                N   L   S   W72  G   D   V
S72D(s)    5'-CA-AAC-CTG-AGC-GAC-GGC-GAT-GTG-GC-3'
S72D(as)   3'-GT-TTG-GAC-TCG-CTG-CCG-CTA-CAC-CG-5'
                N   L   S   D72  G   D   V
S72V(s)    5'-CA-AAC-CTG-AGC-GTT-GGC-GAT-GTG-GC-3'
S72V(as)   3'-GT-TTG-GAC-TCG-CAA-CCG-CTA-CAC-CG-5'
                N   L   S   V72  G   D   V
S72E(s)    5'-CA-AAC-CTG-AGC-GAA-GGC-GAT-GTG-GC-3'
S72E(as)   3'-GT-TTG-GAC-TCG-CTT-CCG-CTA-CAC-CG-5'
                N   L   S   E72  G   D   V
S72M(s)    5'-CA-AAC-CTG-AGC-ATG-GGC-GAT-GTG-GC-3'
S72M(as)   3'-GT-TTG-GAC-TCG-TAC-CCG-CTA-CAC-CG-5'
                N   L   S   M72  G   D   V
S72T(s)    5'-CA-AAC-CTG-AGC-ACC-GGC-GAT-GTG-GC-3'
S72T(as)   3'-GT-TTG-GAC-TCG-TGG-CCG-CTA-CAC-CG-5'
                N   L   S   T72  G   D   V
S72L(s)    5'-CA-AAC-CTG-AGC-CTG-GGC-GAT-GTG-GC-3'
S72L(as)   3'-GT-TTG-GAC-TCG-GAC-CCG-CTA-CAC-CG-5'
                N   L   S   L72  G   D   V
S72R(s)    5'-CA-AAC-CTG-AGC-CGT-GGC-GAT-GTG-GC-3'
S72R(as)   3'-GT-TTG-GAC-TCG-GCA-CCG-CTA-CAC-CG-5'
                N   L   S   R72  G   D   V
S72Q(s)    5'-CA-AAC-CTG-AGC-CAG-GGC-GAT-GTG-GC-3'
S72Q(as)   3'-GT-TTG-GAC-TCG-GTC-CCG-CTA-CAC-CG-5'
                N   L   S   Q72  G   D   V
S72K(s)    5'-CA-AAC-CTG-AGC-AAA-GGC-GAT-GTG-GC-3'
S72K(as)   3'-GT-TTG-GAC-TCG-TTT-CCG-CTA-CAC-CG-5'
                N   L   S   K72  G   D   V
S72P(s)    5'-CA-AAC-CTG-AGC-CCG-GGC-GAT-GTG-GC-3'
S72P(as)   3'-GT-TTG-GAC-TCG-GGC-CCG-CTA-CAC-CG-5'
                N   L   S   P72  G   D   V
S72A(s)    5'-CA-AAC-CTG-AGC-GCG-GGC-GAT-GTG-GC-3'
S72A(as)   3'-GT-TTG-GAC-TCG-CGC-CCG-CTA-CAC-CG-5'
                N   L   S   A72  G   D   V
S72N(s)    5'-CA-AAC-CTG-AGC-AAC-GGC-GAT-GTG-GC-3'
S72N(as)   3'-GT-TTG-GAC-TCG-TTG-CCG-CTA-CAC-CG-5'
                N   L   S   N72  G   D   V
S72G(s)    5'-CA-AAC-CTG-AGC-GGT-GGC-GAT-GTG-GC-3'
S72G(as)   3'-GT-TTG-GAC-TCG-CCA-CCG-CTA-CAC-CG-5'
                N   L   S   G72  G   D   V
S72H(s)    5'-CA-AAC-CTG-AGC-CAC-GGC-GAT-GTG-GC-3'
S72H(as)   3'-GT-TTG-GAC-TCG-GTG-CCG-CTA-CAC-CG-5'
                N   L   S   H72  G   D   V
```

【図８】

```
L16W(s)    5′-CG-AAA-CCG-GAT-TGG-TAC-TAC-CTC-AA-3′
L16W(as)   3′-GC-TTT-GGC-CTA-ACC-ATG-ATG-GAG-TT-5′
                  K   P   D   W16 Y   Y   L

S71W(s)    5′-AT-GCA-AAC-CTG-TGG-AGT-GGC-GAT-GT-3′
S71W(as)   3′-TA-CGT-TTG-GAC-ACC-TCA-CCG-CTA-CA-5′
                  A   N   L   W71 S   G   D

G73W(s)    5′-AC-CTG-AGC-AGT-TGG-GAT-GTG-GCG-AA-3′
G73W(as)   3′-TG-GAC-TCG-TCA-ACC-CTA-CAC-CGC-TT-5′
                  L   S   S   W73 D   V   A

E104F(s)   5′-CC-AAT-ATG-ATT-TTT-GAC-GCC-GGG-GA-3′
E104F(as)  3′-GG-TTA-TAC-TAA-AAA-CTG-CGG-CCC-CT-5′
                  N   M   I   F104 D   A   G

E104W(s)   5′-CC-AAT-ATG-ATT-TGG-GAC-GCC-GGG-GA-3′
E104W(as)  3′-GG-TTA-TAC-TAA-ACC-CTG-CGG-CCC-CT-5′
                  N   M   I   W104 D   A   G
```

【図９】

```
EB-AP:     LALVATGNDTTTKPDLYYLKNSEAINSLALLPPPPAVGSIAFLNDQAMYEQGRLLRNTER
             V  GND TTKPDLYYLKN++AI+SLALLPPPP VGSIAFLNDQAMYE+GRLLRNTER
EA-AP:       LVPAGNDATTKPDLYYLKNAQAIDSLALLPPPPEVGSIAFLNDQAMYEKGRLLRNTER

                      [72]
EB-AP:     GKLAAEDANLSSGGVANAFSGAFGSPITEKDAPALHKLLTNMIEDAGDLATRSAKDHYMR
           GKLAAEDANLS+GGVANAFS AFGSPITEKDAP LHKLLTNMIEDAGDLATRSAK+ YMR
EA-AP:     GKLAAEDANLSAGGVANAFSSAFGSPITEKDAPQLHKLLTNMIEDAGDLATRSAKEKYMR
                     [70]

EB-AP:     IRPFAFYGVSTCNTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELG
           IRPFAFYGVSTCNTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELG
EA-AP:     IRPFAFYGVSTCNTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELG

EB-AP:     QSRVICGYHWQSDVDAARVVGSAVVATLHTNPAFQQQLQKAKAEFAQHQKK
           +SRVICGYHWQSDVDAAR+VGSAVVATLHTNPAFQQQLQKAK EFA+ QK
EA-AP:     ESRVICGYHWQSDVDAARIVGSAVVATLHTNPAFQQQLQKAKDEFAKTQK
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【図１０】

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ATOM      1  N   GLY A   7      35.965  71.208  89.712  1.00 36.57
ATOM      2  CA  GLY A   7      37.459  71.295  89.574  1.00 31.92
ATOM      3  C   GLY A   7      38.160  69.982  89.872  1.00 29.76
ATOM      4  O   GLY A   7      39.301  69.858  89.492  1.00 31.81
ATOM      5  N   ASN A   8      37.485  68.990  90.532  1.00 26.40
ATOM      6  CA  ASN A   8      38.284  67.775  90.697  1.00 26.63
ATOM      7  C   ASN A   8      38.466  67.018  89.396  1.00 29.21
ATOM      8  O   ASN A   8      37.736  67.238  88.431  1.00 30.52
ATOM      9  CB  ASN A   8      37.677  66.810  91.702  1.00 27.01
ATOM     10  CG  ASN A   8      37.725  67.396  93.104  1.00 32.45
ATOM     11  OD1 ASN A   8      38.751  67.744  93.636  1.00 30.02
ATOM     12  ND2 ASN A   8      36.545  67.536  93.707  1.00 31.60
ATOM     13  N   ASP A   9      39.455  66.154  89.463  1.00 29.14
ATOM     14  CA  ASP A   9      39.787  65.216  88.391  1.00 30.47
ATOM     15  C   ASP A   9      40.661  64.081  88.901  1.00 31.02
ATOM     16  O   ASP A   9      40.804  63.931  90.110  1.00 31.00
ATOM     17  CB  ASP A   9      40.394  65.960  87.195  1.00 30.92
ATOM     18  CG  ASP A   9      41.802  66.484  87.429  1.00 32.66
ATOM     19  OD1 ASP A   9      42.307  66.333  88.532  1.00 35.03
ATOM     20  OD2 ASP A   9      42.400  67.018  86.493  1.00 31.63
ATOM     21  N   THR A  10      41.272  63.298  87.998  1.00 28.72
ATOM     22  CA  THR A  10      42.188  62.228  88.430  1.00 28.53
ATOM     23  C   THR A  10      43.408  62.655  89.259  1.00 30.10
ATOM     24  O   THR A  10      43.946  61.944  90.095  1.00 29.06
ATOM     25  CB  THR A  10      42.692  61.405  87.235  1.00 26.05
ATOM     26  OG1 THR A  10      43.272  60.172  87.655  1.00 27.75
ATOM     27  CG2 THR A  10      43.670  62.174  86.313  1.00 23.76
ATOM     28  N   THR A  11      43.814  63.900  88.996  1.00 30.82
ATOM     29  CA  THR A  11      44.932  64.389  89.799  1.00 32.79
ATOM     30  C   THR A  11      44.605  64.736  91.267  1.00 36.32
ATOM     31  O   THR A  11      45.435  64.658  92.162  1.00 37.21
ATOM     32  CB  THR A  11      45.588  65.591  89.143  1.00 30.53
ATOM     33  OG1 THR A  11      44.845  66.781  89.359  1.00 27.79
ATOM     34  CG2 THR A  11      45.899  65.362  87.656  1.00 32.16
ATOM     35  N   THR A  12      43.317  65.076  91.495  1.00 34.81
ATOM     36  CA  THR A  12      42.910  65.213  92.900  1.00 32.91
ATOM     37  C   THR A  12      42.265  63.992  93.549  1.00 33.08
ATOM     38  O   THR A  12      42.350  63.742  94.736  1.00 32.49
ATOM     39  CB  THR A  12      41.963  66.395  93.077  1.00 30.92
ATOM     40  OG1 THR A  12      40.719  66.162  92.409  1.00 32.04
ATOM     41  CG2 THR A  12      42.599  67.667  92.543  1.00 29.75
ATOM     42  N   LYS A  13      41.565  63.229  92.703  1.00 31.17
ATOM     43  CA  LYS A  13      40.791  62.064  93.174  1.00 30.27
ATOM     44  C   LYS A  13      40.904  60.812  92.287  1.00 31.40
ATOM     45  O   LYS A  13      39.981  60.348  91.605  1.00 33.05
ATOM     46  CB  LYS A  13      39.294  62.395  93.331  1.00 29.09
ATOM     47  CG  LYS A  13      39.001  63.747  93.965  1.00 32.97
ATOM     48  CD  LYS A  13      37.536  64.076  94.166  1.00 37.86
ATOM     49  CE  LYS A  13      36.767  62.909  94.772  1.00 47.28
ATOM     50  NZ  LYS A  13      35.340  63.270  94.947  1.00 52.08
ATOM     51  N   PRO A  14      42.138  60.283  92.279  1.00 33.01
ATOM     52  CA  PRO A  14      42.516  59.249  91.290  1.00 32.06
ATOM     53  C   PRO A  14      41.823  57.907  91.452  1.00 30.98
ATOM     54  O   PRO A  14      41.961  56.989  90.668  1.00 32.57
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【図１１】

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ATOM     55  CB  PRO A  14      44.035  59.145  91.468  1.00 34.46
ATOM     56  CG  PRO A  14      44.283  59.564  92.920  1.00 33.02
ATOM     57  CD  PRO A  14      43.225  60.638  93.181  1.00 34.46
ATOM     58  N   ASP A  15      41.046  57.815  92.513  1.00 29.27
ATOM     59  CA  ASP A  15      40.204  56.655  92.809  1.00 28.89
ATOM     60  C   ASP A  15      38.810  56.684  92.146  1.00 21.76
ATOM     61  O   ASP A  15      38.078  55.706  92.030  1.00 20.59
ATOM     62  CB  ASP A  15      40.125  56.599  94.368  1.00 37.60
ATOM     63  CG  ASP A  15      39.589  57.903  95.080  1.00 45.11
ATOM     64  OD1 ASP A  15      40.062  59.044  94.817  1.00 45.67
ATOM     65  OD2 ASP A  15      38.687  57.751  95.922  1.00 49.07
ATOM     66  N   LEU A  16      38.495  57.910  91.726  1.00 20.49
ATOM     67  CA  LEU A  16      37.182  58.179  91.135  1.00 23.90
ATOM     68  C   LEU A  16      37.156  58.814  89.727  1.00 22.23
ATOM     69  O   LEU A  16      36.109  59.011  89.134  1.00 23.21
ATOM     70  CB  LEU A  16      36.354  59.099  92.029  1.00 23.35
ATOM     71  CG  LEU A  16      35.814  58.432  93.297  1.00 25.48
ATOM     72  CD1 LEU A  16      34.876  57.253  93.075  1.00 24.05
ATOM     73  CD2 LEU A  16      35.092  59.477  94.104  1.00 25.22
ATOM     74  N   TYR A  17      38.343  59.175  89.273  1.00 20.96
ATOM     75  CA  TYR A  17      38.555  59.605  87.889  1.00 22.04
ATOM     76  C   TYR A  17      39.780  58.903  87.334  1.00 22.80
ATOM     77  O   TYR A  17      40.790  58.799  88.021  1.00 23.48
ATOM     78  CB  TYR A  17      38.856  61.095  87.711  1.00 18.01
ATOM     79  CG  TYR A  17      37.928  62.099  88.371  1.00 24.78
ATOM     80  CD1 TYR A  17      37.129  62.916  87.542  1.00 22.78
ATOM     81  CD2 TYR A  17      37.905  62.248  89.781  1.00 23.58
ATOM     82  CE1 TYR A  17      36.317  63.919  88.113  1.00 26.51
ATOM     83  CE2 TYR A  17      37.090  63.240  90.349  1.00 22.88
ATOM     84  CZ  TYR A  17      36.303  64.059  89.517  1.00 24.63
ATOM     85  OH  TYR A  17      35.482  65.023  90.066  1.00 22.92
ATOM     86  N   TYR A  18      39.670  58.482  86.053  1.00 26.17
ATOM     87  CA  TYR A  18      40.838  58.209  85.191  1.00 21.13
ATOM     88  C   TYR A  18      41.332  59.414  84.464  1.00 19.92
ATOM     89  O   TYR A  18      42.490  59.511  84.083  1.00 22.64
ATOM     90  CB  TYR A  18      40.563  57.195  84.080  1.00 17.53
ATOM     91  CG  TYR A  18      40.312  55.826  84.610  1.00 16.91
ATOM     92  CD1 TYR A  18      41.425  55.028  84.916  1.00 19.86
ATOM     93  CD2 TYR A  18      38.985  55.372  84.771  1.00 16.65
ATOM     94  CE1 TYR A  18      41.218  53.725  85.383  1.00 18.64
ATOM     95  CE2 TYR A  18      38.765  54.053  85.213  1.00 17.52
ATOM     96  CZ  TYR A  18      39.892  53.262  85.515  1.00 21.18
ATOM     97  OH  TYR A  18      39.734  51.974  85.977  1.00 26.15
ATOM     98  N   LEU A  19      40.412  60.336  84.236  1.00 21.49
ATOM     99  CA  LEU A  19      40.788  61.462  83.366  1.00 22.71
ATOM    100  C   LEU A  19      41.094  62.812  84.021  1.00 25.01
ATOM    101  O   LEU A  19      40.771  63.125  85.159  1.00 25.24
ATOM    102  CB  LEU A  19      39.708  61.669  82.290  1.00 21.68
ATOM    103  CG  LEU A  19      39.301  60.442  81.432  1.00 22.88
ATOM    104  CD1 LEU A  19      40.430  59.842  80.583  1.00 20.39
ATOM    105  CD2 LEU A  19      38.078  60.812  80.608  1.00 18.83
ATOM    106  N   LYS A  20      41.736  63.667  83.246  1.00 26.44
ATOM    107  CA  LYS A  20      41.947  65.032  83.717  1.00 26.77
ATOM    108  C   LYS A  20      40.935  66.034  83.292  1.00 26.42
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ATOM    109  O   LYS A  20      40.182  65.870  82.341  1.00 29.05
ATOM    110  CB  LYS A  20      43.239  65.608  83.187  1.00 30.61
ATOM    111  CG  LYS A  20      44.400  64.791  83.648  1.00 32.90
ATOM    112  CD  LYS A  20      45.633  65.326  82.963  1.00 39.72
ATOM    113  CE  LYS A  20      46.698  64.259  83.113  1.00 50.27
ATOM    114  NZ  LYS A  20      46.148  62.977  82.610  1.00 62.00
ATOM    115  N   ASN A  21      41.050  67.184  83.943  1.00 24.09
ATOM    116  CA  ASN A  21      40.154  68.246  83.530  1.00 23.98
ATOM    117  C   ASN A  21      40.177  68.539  82.032  1.00 25.08
ATOM    118  O   ASN A  21      39.134  68.722  81.427  1.00 25.36
ATOM    119  CB  ASN A  21      40.310  69.512  84.371  1.00 23.81
ATOM    120  CG  ASN A  21      39.601  69.311  85.697  1.00 26.97
ATOM    121  OD1 ASN A  21      38.392  69.175  85.836  1.00 26.36
ATOM    122  ND2 ASN A  21      40.403  69.303  86.744  1.00 32.00
ATOM    123  N   SER A  22      41.378  68.486  81.450  1.00 23.72
ATOM    124  CA  SER A  22      41.592  68.804  80.008  1.00 25.53
ATOM    125  C   SER A  22      40.992  67.752  79.068  1.00 25.77
ATOM    126  O   SER A  22      40.524  68.007  77.966  1.00 27.65
ATOM    127  CB  SER A  22      43.079  68.868  79.699  1.00 23.34
ATOM    128  OG  SER A  22      43.719  67.716  80.303  1.00 33.30
ATOM    129  N   GLU A  23      40.957  66.529  79.624  1.00 22.35
ATOM    130  CA  GLU A  23      40.320  65.466  78.899  1.00 21.87
ATOM    131  C   GLU A  23      38.811  65.375  78.974  1.00 23.18
ATOM    132  O   GLU A  23      38.197  64.451  78.471  1.00 25.83
ATOM    133  CB  GLU A  23      40.923  64.165  79.337  1.00 22.13
ATOM    134  CG  GLU A  23      42.451  64.215  79.214  1.00 26.78
ATOM    135  CD  GLU A  23      43.021  62.908  79.718  1.00 30.40
ATOM    136  OE1 GLU A  23      42.946  62.648  80.900  1.00 31.10
ATOM    137  OE2 GLU A  23      43.544  62.118  78.957  1.00 32.35
ATOM    138  N   ALA A  24      38.196  66.359  79.610  1.00 21.49
ATOM    139  CA  ALA A  24      36.751  66.165  79.738  1.00 22.48
ATOM    140  C   ALA A  24      35.973  66.420  78.438  1.00 22.81
ATOM    141  O   ALA A  24      36.325  67.333  77.704  1.00 23.36
ATOM    142  CB  ALA A  24      36.188  67.183  80.734  1.00 21.43
ATOM    143  N   ILE A  25      34.859  65.694  78.228  1.00 23.46
ATOM    144  CA  ILE A  25      33.845  66.149  77.243  1.00 23.60
ATOM    145  C   ILE A  25      33.312  67.535  77.530  1.00 24.71
ATOM    146  O   ILE A  25      32.788  67.809  78.603  1.00 25.03
ATOM    147  CB  ILE A  25      32.684  65.160  77.096  1.00 20.26
ATOM    148  CG1 ILE A  25      33.237  63.749  76.838  1.00 23.14
ATOM    149  CG2 ILE A  25      31.739  65.555  75.954  1.00 21.26
ATOM    150  CD1 ILE A  25      34.298  63.551  75.722  1.00 16.94
ATOM    151  N   ASN A  26      33.485  68.431  76.562  1.00 22.50
ATOM    152  CA  ASN A  26      32.797  69.706  76.751  1.00 22.04
ATOM    153  C   ASN A  26      31.295  69.680  76.533  1.00 22.52
ATOM    154  O   ASN A  26      30.731  70.042  75.509  1.00 22.34
ATOM    155  CB  ASN A  26      33.474  70.744  75.900  1.00 20.15
ATOM    156  CG  ASN A  26      32.982  72.133  76.217  1.00 24.94
ATOM    157  OD1 ASN A  26      31.923  72.459  76.732  1.00 29.46
ATOM    158  ND2 ASN A  26      33.827  73.032  75.809  1.00 25.76
ATOM    159  N   SER A  27      30.627  69.289  77.622  1.00 19.77
ATOM    160  CA  SER A  27      29.166  69.168  77.549  1.00 18.88
ATOM    161  C   SER A  27      28.412  70.423  77.177  1.00 18.74
ATOM    162  O   SER A  27      27.390  70.393  76.517  1.00 21.73
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ATOM	163	CB	SER	A	27	28.606	68.619	78.870	1.00	19.35
ATOM	164	OG	SER	A	27	28.967	69.518	79.940	1.00	19.36
ATOM	165	N	LEU	A	28	28.961	71.564	77.588	1.00	18.08
ATOM	166	CA	LEU	A	28	28.271	72.815	77.262	1.00	20.15
ATOM	167	C	LEU	A	28	28.283	73.062	75.761	1.00	23.12
ATOM	168	O	LEU	A	28	27.303	73.485	75.165	1.00	22.25
ATOM	169	CB	LEU	A	28	28.990	74.042	77.798	1.00	17.00
ATOM	170	CG	LEU	A	28	28.159	75.188	78.376	1.00	18.01
ATOM	171	CD1	LEU	A	28	26.847	75.547	77.733	1.00	14.28
ATOM	172	CD2	LEU	A	28	29.053	76.394	78.592	1.00	16.45
ATOM	173	N	ALA	A	29	29.478	72.767	75.193	1.00	23.87
ATOM	174	CA	ALA	A	29	29.598	72.827	73.707	1.00	22.62
ATOM	175	C	ALA	A	29	28.773	71.847	72.837	1.00	20.86
ATOM	176	O	ALA	A	29	28.192	72.239	71.830	1.00	25.89
ATOM	177	CB	ALA	A	29	31.065	72.692	73.326	1.00	20.24
ATOM	178	N	LEU	A	30	28.733	70.580	73.267	1.00	15.78
ATOM	179	CA	LEU	A	30	28.079	69.497	72.519	1.00	18.05
ATOM	180	C	LEU	A	30	26.557	69.416	72.559	1.00	22.38
ATOM	181	O	LEU	A	30	25.845	69.251	71.566	1.00	23.63
ATOM	182	CB	LEU	A	30	28.732	68.194	72.977	1.00	16.47
ATOM	183	CG	LEU	A	30	28.234	66.887	72.360	1.00	17.59
ATOM	184	CD1	LEU	A	30	28.812	65.706	73.120	1.00	12.95
ATOM	185	CD2	LEU	A	30	28.456	66.775	70.850	1.00	13.89
ATOM	186	N	LEU	A	31	26.075	69.533	73.812	1.00	22.39
ATOM	187	CA	LEU	A	31	24.633	69.430	74.049	1.00	18.84
ATOM	188	C	LEU	A	31	23.817	70.624	73.538	1.00	16.30
ATOM	189	O	LEU	A	31	24.260	71.763	73.576	1.00	19.93
ATOM	190	CB	LEU	A	31	24.381	69.199	75.556	1.00	16.94
ATOM	191	CG	LEU	A	31	24.923	67.873	76.095	1.00	17.95
ATOM	192	CD1	LEU	A	31	24.177	66.669	75.553	1.00	11.60
ATOM	193	CD2	LEU	A	31	24.823	67.878	77.628	1.00	18.77
ATOM	194	N	PRO	A	32	22.581	70.333	73.105	1.00	14.80
ATOM	195	CA	PRO	A	32	21.589	71.404	72.910	1.00	18.31
ATOM	196	C	PRO	A	32	21.228	72.028	74.278	1.00	22.24
ATOM	197	O	PRO	A	32	21.453	71.442	75.327	1.00	22.27
ATOM	198	CB	PRO	A	32	20.402	70.621	72.348	1.00	15.94
ATOM	199	CG	PRO	A	32	20.545	69.184	72.847	1.00	16.93
ATOM	200	CD	PRO	A	32	22.038	68.972	72.954	1.00	15.56
ATOM	201	N	PRO	A	33	20.657	73.249	74.287	1.00	23.41
ATOM	202	CA	PRO	A	33	20.190	73.780	75.586	1.00	20.34
ATOM	203	C	PRO	A	33	19.059	72.945	76.084	1.00	19.93
ATOM	204	O	PRO	A	33	18.409	72.292	75.285	1.00	18.67
ATOM	205	CB	PRO	A	33	19.659	75.158	75.224	1.00	18.52
ATOM	206	CG	PRO	A	33	20.267	75.499	73.877	1.00	21.71
ATOM	207	CD	PRO	A	33	20.406	74.146	73.177	1.00	21.12
ATOM	208	N	PRO	A	34	18.785	72.950	77.411	1.00	19.36
ATOM	209	CA	PRO	A	34	17.645	72.138	77.863	1.00	13.70
ATOM	210	C	PRO	A	34	16.348	72.759	77.351	1.00	11.77
ATOM	211	O	PRO	A	34	16.280	73.937	77.090	1.00	14.58
ATOM	212	CB	PRO	A	34	17.760	72.358	79.389	1.00	13.66
ATOM	213	CG	PRO	A	34	18.471	73.698	79.571	1.00	14.81
ATOM	214	CD	PRO	A	34	19.499	73.679	78.464	1.00	16.49
ATOM	215	N	PRO	A	35	15.257	72.007	77.284	1.00	12.52
ATOM	216	CA	PRO	A	35	14.011	72.710	76.973	1.00	13.71

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ATOM    217  C   PRO A  35      13.665  73.842  77.945  1.00 20.26
ATOM    218  O   PRO A  35      13.728  73.715  79.159  1.00 20.52
ATOM    219  CB  PRO A  35      12.997  71.579  76.991  1.00 11.74
ATOM    220  CG  PRO A  35      13.723  70.243  77.051  1.00 12.04
ATOM    221  CD  PRO A  35      15.140  70.581  77.482  1.00 11.57
ATOM    222  N   ALA A  36      13.311  74.962  77.356  1.00 19.25
ATOM    223  CA  ALA A  36      12.919  76.136  78.122  1.00 18.78
ATOM    224  C   ALA A  36      11.457  76.120  78.497  1.00 18.25
ATOM    225  O   ALA A  36      10.582  75.579  77.847  1.00 18.88
ATOM    226  CB  ALA A  36      13.152  77.414  77.304  1.00 17.95
ATOM    227  N   VAL A  37      11.182  76.753  79.609  1.00 18.03
ATOM    228  CA  VAL A  37       9.803  77.005  79.965  1.00 16.78
ATOM    229  C   VAL A  37       9.135  77.993  78.998  1.00 17.18
ATOM    230  O   VAL A  37       9.640  79.048  78.650  1.00 19.89
ATOM    231  CB  VAL A  37       9.740  77.500  81.436  1.00 18.33
ATOM    232  CG1 VAL A  37      10.381  76.501  82.418  1.00 13.83
ATOM    233  CG2 VAL A  37       8.300  77.825  81.832  1.00 14.21
ATOM    234  N   GLY A  38       7.952  77.616  78.561  1.00 18.34
ATOM    235  CA  GLY A  38       7.422  78.249  77.343  1.00 22.06
ATOM    236  C   GLY A  38       7.538  77.398  76.043  1.00 21.25
ATOM    237  O   GLY A  38       6.851  77.623  75.068  1.00 22.09
ATOM    238  N   SER A  39       8.422  76.401  76.060  1.00 21.73
ATOM    239  CA  SER A  39       8.520  75.487  74.905  1.00 20.30
ATOM    240  C   SER A  39       7.604  74.277  74.964  1.00 21.10
ATOM    241  O   SER A  39       7.217  73.736  76.002  1.00 19.55
ATOM    242  CB  SER A  39       9.946  74.998  74.748  1.00 15.45
ATOM    243  OG  SER A  39      10.197  73.967  75.704  1.00 15.38
ATOM    244  N   ILE A  40       7.287  73.796  73.772  1.00 17.17
ATOM    245  CA  ILE A  40       6.618  72.485  73.702  1.00 14.71
ATOM    246  C   ILE A  40       7.475  71.311  74.225  1.00 10.81
ATOM    247  O   ILE A  40       6.998  70.315  74.782  1.00 15.23
ATOM    248  CB  ILE A  40       6.102  72.235  72.219  1.00 15.78
ATOM    249  CG1 ILE A  40       5.162  73.368  71.791  1.00 15.41
ATOM    250  CG2 ILE A  40       5.406  70.863  72.091  1.00 14.54
ATOM    251  CD1 ILE A  40       4.812  73.332  70.307  1.00 18.26
ATOM    252  N   ALA A  41       8.790  71.443  74.040  1.00 10.69
ATOM    253  CA  ALA A  41       9.633  70.373  74.530  1.00 13.79
ATOM    254  C   ALA A  41       9.566  70.300  76.091  1.00 15.36
ATOM    255  O   ALA A  41       9.369  69.245  76.683  1.00 20.02
ATOM    256  CB  ALA A  41      11.046  70.610  74.065  1.00 11.61
ATOM    257  N   PHE A  42       9.547  71.495  76.702  1.00 16.94
ATOM    258  CA  PHE A  42       9.200  71.480  78.151  1.00 15.75
ATOM    259  C   PHE A  42       7.818  70.970  78.533  1.00 16.07
ATOM    260  O   PHE A  42       7.652  70.182  79.448  1.00 19.72
ATOM    261  CB  PHE A  42       9.513  72.819  78.819  1.00 17.93
ATOM    262  CG  PHE A  42       9.380  72.700  80.338  1.00 20.96
ATOM    263  CD1 PHE A  42      10.297  71.904  81.056  1.00 19.46
ATOM    264  CD2 PHE A  42       8.324  73.370  80.997  1.00 20.99
ATOM    265  CE1 PHE A  42      10.148  71.763  82.450  1.00 17.30
ATOM    266  CE2 PHE A  42       8.190  73.248  82.402  1.00 19.79
ATOM    267  CZ  PHE A  42       9.111  72.443  83.100  1.00 13.36
ATOM    268  N   LEU A  43       6.790  71.375  77.765  1.00 19.00
ATOM    269  CA  LEU A  43       5.507  70.643  77.917  1.00 19.22
ATOM    270  C   LEU A  43       5.573  69.103  77.945  1.00 19.39
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ATOM    271  O   LEU A  43       4.957  68.410  78.749  1.00 17.69
ATOM    272  CB  LEU A  43       4.472  71.003  76.826  1.00 21.60
ATOM    273  CG  LEU A  43       3.213  71.850  77.034  1.00 24.67
ATOM    274  CD1 LEU A  43       2.597  71.800  78.433  1.00 17.68
ATOM    275  CD2 LEU A  43       2.172  71.549  75.953  1.00 21.72
ATOM    276  N   ASN A  44       6.392  68.588  77.023  1.00 19.47
ATOM    277  CA  ASN A  44       6.653  67.176  77.076  1.00 19.82
ATOM    278  C   ASN A  44       7.419  66.619  78.312  1.00 17.44
ATOM    279  O   ASN A  44       7.018  65.604  78.855  1.00 15.52
ATOM    280  CB  ASN A  44       7.259  66.847  75.747  1.00 19.07
ATOM    281  CG  ASN A  44       7.491  65.366  75.643  1.00 22.97
ATOM    282  OD1 ASN A  44       8.605  64.906  75.468  1.00 30.35
ATOM    283  ND2 ASN A  44       6.444  64.588  75.862  1.00 22.13
ATOM    284  N   ASP A  45       8.482  67.324  78.726  1.00 19.71
ATOM    285  CA  ASP A  45       9.175  67.050  80.020  1.00 19.87
ATOM    286  C   ASP A  45       8.192  66.977  81.213  1.00 19.30
ATOM    287  O   ASP A  45       8.103  66.009  81.956  1.00 21.00
ATOM    288  CB  ASP A  45      10.225  68.119  80.273  1.00 13.57
ATOM    289  CG  ASP A  45      11.563  67.769  79.706  1.00 12.64
ATOM    290  OD1 ASP A  45      12.408  68.656  79.625  1.00 15.68
ATOM    291  OD2 ASP A  45      11.823  66.611  79.414  1.00 14.57
ATOM    292  N   GLN A  46       7.347  68.007  81.299  1.00 19.62
ATOM    293  CA  GLN A  46       6.199  67.904  82.220  1.00 19.44
ATOM    294  C   GLN A  46       5.259  66.702  82.166  1.00 22.23
ATOM    295  O   GLN A  46       4.960  66.057  83.175  1.00 21.67
ATOM    296  CB  GLN A  46       5.353  69.153  82.218  1.00 16.35
ATOM    297  CG  GLN A  46       6.282  70.333  82.395  1.00 18.35
ATOM    298  CD  GLN A  46       5.398  71.519  82.591  1.00 26.07
ATOM    299  OE1 GLN A  46       5.334  72.143  83.629  1.00 31.83
ATOM    300  NE2 GLN A  46       4.622  71.823  81.591  1.00 22.82
ATOM    301  N   ALA A  47       4.838  66.364  80.935  1.00 19.12
ATOM    302  CA  ALA A  47       3.979  65.187  80.813  1.00 17.83
ATOM    303  C   ALA A  47       4.661  63.871  81.172  1.00 15.90
ATOM    304  O   ALA A  47       4.065  62.940  81.701  1.00 18.55
ATOM    305  CB  ALA A  47       3.441  65.066  79.367  1.00 17.11
ATOM    306  N   MET A  48       5.970  63.818  80.841  1.00 18.16
ATOM    307  CA  MET A  48       6.799  62.644  81.235  1.00 19.52
ATOM    308  C   MET A  48       7.012  62.460  82.765  1.00 21.38
ATOM    309  O   MET A  48       6.996  61.358  83.316  1.00 20.83
ATOM    310  CB  MET A  48       8.173  62.667  80.539  1.00 21.42
ATOM    311  CG  MET A  48       8.150  62.603  78.984  1.00 29.81
ATOM    312  SD  MET A  48       7.330  61.126  78.308  1.00 36.20
ATOM    313  CE  MET A  48       5.582  61.633  78.280  1.00 33.60
ATOM    314  N   TYR A  49       7.139  63.655  83.414  1.00 21.32
ATOM    315  CA  TYR A  49       7.066  63.807  84.885  1.00 21.30
ATOM    316  C   TYR A  49       5.773  63.244  85.515  1.00 22.58
ATOM    317  O   TYR A  49       5.797  62.383  86.390  1.00 24.04
ATOM    318  CB  TYR A  49       7.304  65.282  85.217  1.00 20.61
ATOM    319  CG  TYR A  49       7.034  65.494  86.692  1.00 23.57
ATOM    320  CD1 TYR A  49       5.755  65.931  87.109  1.00 23.57
ATOM    321  CD2 TYR A  49       8.080  65.194  87.574  1.00 21.83
ATOM    322  CE1 TYR A  49       5.524  66.097  88.481  1.00 26.09
ATOM    323  CE2 TYR A  49       7.844  65.349  88.943  1.00 23.18
ATOM    324  CZ  TYR A  49       6.591  65.842  89.377  1.00 26.31
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ATOM    325  OH  TYR A  49       6.444  66.124  90.726  1.00 29.46
ATOM    326  N   GLU A  50       4.639  63.731  84.994  1.00 22.09
ATOM    327  CA  GLU A  50       3.336  63.234  85.472  1.00 21.48
ATOM    328  C   GLU A  50       3.052  61.776  85.230  1.00 23.20
ATOM    329  O   GLU A  50       2.548  61.050  86.081  1.00 24.23
ATOM    330  CB  GLU A  50       2.190  64.023  84.862  1.00 21.88
ATOM    331  CG  GLU A  50       2.304  65.537  84.986  1.00 21.13
ATOM    332  CD  GLU A  50       2.054  65.976  86.417  1.00 25.41
ATOM    333  OE1 GLU A  50       1.887  65.138  87.287  1.00 24.65
ATOM    334  OE2 GLU A  50       2.004  67.162  86.679  1.00 25.05
ATOM    335  N   GLN A  51       3.479  61.343  84.032  1.00 23.02
ATOM    336  CA  GLN A  51       3.427  59.907  83.812  1.00 24.72
ATOM    337  C   GLN A  51       4.275  59.006  84.728  1.00 26.23
ATOM    338  O   GLN A  51       3.804  57.996  85.253  1.00 25.10
ATOM    339  CB  GLN A  51       3.680  59.545  82.355  1.00 24.41
ATOM    340  CG  GLN A  51       3.461  58.028  82.141  1.00 38.05
ATOM    341  CD  GLN A  51       2.115  57.435  82.657  1.00 53.15
ATOM    342  OE1 GLN A  51       1.093  58.076  82.867  1.00 61.03
ATOM    343  NE2 GLN A  51       2.098  56.123  82.834  1.00 55.24
ATOM    344  N   GLY A  52       5.556  59.414  84.922  1.00 26.76
ATOM    345  CA  GLY A  52       6.400  58.689  85.876  1.00 26.91
ATOM    346  C   GLY A  52       5.793  58.681  87.286  1.00 25.60
ATOM    347  O   GLY A  52       5.666  57.699  87.997  1.00 24.37
ATOM    348  N   ARG A  53       5.321  59.874  87.621  1.00 28.09
ATOM    349  CA  ARG A  53       4.527  60.032  88.834  1.00 29.75
ATOM    350  C   ARG A  53       3.384  59.049  89.067  1.00 32.01
ATOM    351  O   ARG A  53       3.284  58.437  90.115  1.00 34.69
ATOM    352  CB  ARG A  53       4.128  61.494  88.965  1.00 30.41
ATOM    353  CG  ARG A  53       3.857  61.919  90.389  1.00 29.15
ATOM    354  CD  ARG A  53       3.519  63.393  90.461  1.00 29.38
ATOM    355  NE  ARG A  53       2.385  63.740  89.609  1.00 31.35
ATOM    356  CZ  ARG A  53       1.088  63.593  89.886  1.00 32.29
ATOM    357  NH1 ARG A  53       0.187  64.144  89.125  1.00 32.06
ATOM    358  NH2 ARG A  53       0.661  62.938  90.931  1.00 32.66
ATOM    359  N   LEU A  54       2.575  58.839  88.033  1.00 32.47
ATOM    360  CA  LEU A  54       1.588  57.735  88.076  1.00 31.67
ATOM    361  C   LEU A  54       2.114  56.273  88.119  1.00 33.30
ATOM    362  O   LEU A  54       1.452  55.329  88.568  1.00 35.90
ATOM    363  CB  LEU A  54       0.603  57.880  86.901  1.00 33.52
ATOM    364  CG  LEU A  54      -0.599  58.822  87.055  1.00 32.85
ATOM    365  CD1 LEU A  54      -1.298  59.020  85.709  1.00 30.53
ATOM    366  CD2 LEU A  54      -0.286  60.130  87.777  1.00 36.56
ATOM    367  N   LEU A  55       3.374  56.117  87.657  1.00 31.95
ATOM    368  CA  LEU A  55       4.016  54.784  87.735  1.00 32.70
ATOM    369  C   LEU A  55       4.577  54.392  89.091  1.00 33.98
ATOM    370  O   LEU A  55       4.842  53.224  89.386  1.00 32.73
ATOM    371  CB  LEU A  55       5.194  54.646  86.790  1.00 31.04
ATOM    372  CG  LEU A  55       4.832  54.514  85.343  1.00 28.60
ATOM    373  CD1 LEU A  55       3.960  53.287  85.101  1.00 27.88
ATOM    374  CD2 LEU A  55       6.118  54.471  84.539  1.00 28.33
ATOM    375  N   ARG A  56       4.732  55.458  89.911  1.00 36.20
ATOM    376  CA  ARG A  56       5.257  55.308  91.281  1.00 37.04
ATOM    377  C   ARG A  56       4.616  54.240  92.164  1.00 40.28
ATOM    378  O   ARG A  56       5.260  53.518  92.907  1.00 41.22
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ATOM    379  CB  ARG A  56       5.249  56.643  91.993  1.00 32.58
ATOM    380  CG  ARG A  56       6.368  57.502  91.476  1.00 22.04
ATOM    381  CD  ARG A  56       6.142  58.874  92.049  1.00 21.74
ATOM    382  NE  ARG A  56       7.073  59.804  91.447  1.00 23.56
ATOM    383  CZ  ARG A  56       7.062  61.074  91.750  1.00 25.56
ATOM    384  NH1 ARG A  56       6.401  61.444  92.790  1.00 30.94
ATOM    385  NH2 ARG A  56       7.688  61.979  91.035  1.00 22.33
ATOM    386  N   ASN A  57       3.306  54.120  91.997  1.00 44.42
ATOM    387  CA  ASN A  57       2.602  53.027  92.680  1.00 48.62
ATOM    388  C   ASN A  57       2.786  51.585  92.169  1.00 47.46
ATOM    389  O   ASN A  57       2.316  50.630  92.759  1.00 51.15
ATOM    390  CB  ASN A  57       1.124  53.435  92.726  1.00 59.30
ATOM    391  CG  ASN A  57       0.389  53.137  94.049  1.00 68.98
ATOM    392  OD1 ASN A  57      -0.829  53.335  94.164  1.00 75.09
ATOM    393  ND2 ASN A  57       1.140  52.692  95.058  1.00 71.68
ATOM    394  N   THR A  58       3.461  51.442  91.036  1.00 42.68
ATOM    395  CA  THR A  58       3.555  50.086  90.475  1.00 36.64
ATOM    396  C   THR A  58       4.821  49.318  90.871  1.00 33.64
ATOM    397  O   THR A  58       5.721  49.876  91.477  1.00 31.69
ATOM    398  CB  THR A  58       3.492  50.189  88.948  1.00 36.81
ATOM    399  OG1 THR A  58       4.774  50.576  88.447  1.00 37.64
ATOM    400  CG2 THR A  58       2.432  51.203  88.507  1.00 35.36
ATOM    401  N   GLU A  59       4.937  48.068  90.409  1.00 33.08
ATOM    402  CA  GLU A  59       6.238  47.410  90.581  1.00 34.80
ATOM    403  C   GLU A  59       7.487  48.104  89.944  1.00 33.45
ATOM    404  O   GLU A  59       8.607  48.153  90.463  1.00 34.28
ATOM    405  CB  GLU A  59       6.067  45.933  90.191  1.00 43.43
ATOM    406  CG  GLU A  59       7.242  45.007  90.614  1.00 59.74
ATOM    407  CD  GLU A  59       7.519  44.933  92.159  1.00 69.61
ATOM    408  OE1 GLU A  59       6.582  45.064  92.960  1.00 74.78
ATOM    409  OE2 GLU A  59       8.686  44.751  92.589  1.00 74.97
ATOM    410  N   ARG A  60       7.229  48.734  88.768  1.00 27.61
ATOM    411  CA  ARG A  60       8.251  49.599  88.158  1.00 25.02
ATOM    412  C   ARG A  60       8.614  50.851  88.958  1.00 22.94
ATOM    413  O   ARG A  60       9.772  51.257  89.002  1.00 24.63
ATOM    414  CB  ARG A  60       7.874  49.966  86.690  1.00 26.16
ATOM    415  CG  ARG A  60       8.877  50.860  85.900  1.00 24.47
ATOM    416  CD  ARG A  60      10.268  50.249  85.758  1.00 23.96
ATOM    417  NE  ARG A  60      11.285  51.161  85.217  1.00 25.64
ATOM    418  CZ  ARG A  60      12.214  51.778  85.945  1.00 24.77
ATOM    419  NH1 ARG A  60      12.159  51.805  87.261  1.00 24.78
ATOM    420  NH2 ARG A  60      13.227  52.294  85.325  1.00 19.79
ATOM    421  N   GLY A  61       7.562  51.411  89.587  1.00 21.94
ATOM    422  CA  GLY A  61       7.623  52.443  90.620  1.00 22.33
ATOM    423  C   GLY A  61       8.468  52.051  91.824  1.00 24.44
ATOM    424  O   GLY A  61       9.350  52.773  92.253  1.00 25.22
ATOM    425  N   LYS A  62       8.248  50.821  92.307  1.00 26.95
ATOM    426  CA  LYS A  62       9.102  50.251  93.350  1.00 26.24
ATOM    427  C   LYS A  62      10.590  50.158  93.045  1.00 24.89
ATOM    428  O   LYS A  62      11.443  50.668  93.756  1.00 23.23
ATOM    429  CB  LYS A  62       8.519  48.900  93.723  1.00 29.90
ATOM    430  CG  LYS A  62       9.379  48.296  94.835  1.00 38.76
ATOM    431  CD  LYS A  62       8.847  46.904  95.222  1.00 47.47
ATOM    432  CE  LYS A  62       9.944  45.971  95.773  1.00 53.72
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ATOM    433  NZ  LYS A  62      10.167  44.857  94.832  1.00 60.22
ATOM    434  N   LEU A  63      10.866  49.560  91.882  1.00 24.67
ATOM    435  CA  LEU A  63      12.239  49.634  91.346  1.00 23.65
ATOM    436  C   LEU A  63      12.805  51.043  91.186  1.00 22.74
ATOM    437  O   LEU A  63      13.927  51.359  91.517  1.00 25.19
ATOM    438  CB  LEU A  63      12.232  48.973  89.981  1.00 27.11
ATOM    439  CG  LEU A  63      13.477  48.298  89.403  1.00 31.83
ATOM    440  CD1 LEU A  63      14.808  48.658  90.077  1.00 33.84
ATOM    441  CD2 LEU A  63      13.440  48.410  87.874  1.00 26.95
ATOM    442  N   ALA A  64      11.979  51.940  90.642  1.00 21.62
ATOM    443  CA  ALA A  64      12.492  53.308  90.539  1.00 20.51
ATOM    444  C   ALA A  64      12.862  53.971  91.863  1.00 21.79
ATOM    445  O   ALA A  64      13.890  54.636  91.984  1.00 21.21
ATOM    446  CB  ALA A  64      11.456  54.196  89.862  1.00 19.01
ATOM    447  N   ALA A  65      11.984  53.747  92.870  1.00 23.25
ATOM    448  CA  ALA A  65      12.374  54.212  94.235  1.00 24.88
ATOM    449  C   ALA A  65      13.684  53.619  94.784  1.00 22.62
ATOM    450  O   ALA A  65      14.551  54.328  95.268  1.00 22.95
ATOM    451  CB  ALA A  65      11.249  54.013  95.265  1.00 25.38
ATOM    452  N   GLU A  66      13.848  52.295  94.572  1.00 23.38
ATOM    453  CA  GLU A  66      15.116  51.632  94.878  1.00 23.41
ATOM    454  C   GLU A  66      16.332  52.188  94.152  1.00 26.06
ATOM    455  O   GLU A  66      17.321  52.604  94.744  1.00 25.12
ATOM    456  CB  GLU A  66      14.968  50.136  94.665  1.00 25.85
ATOM    457  CG  GLU A  66      13.818  49.616  95.533  1.00 31.94
ATOM    458  CD  GLU A  66      13.546  48.142  95.293  1.00 37.59
ATOM    459  OE1 GLU A  66      13.147  47.430  96.220  1.00 40.33
ATOM    460  OE2 GLU A  66      13.721  47.673  94.176  1.00 40.79
ATOM    461  N   ASP A  67      16.204  52.276  92.817  1.00 24.01
ATOM    462  CA  ASP A  67      17.222  52.928  91.986  1.00 19.72
ATOM    463  C   ASP A  67      17.549  54.333  92.402  1.00 16.72
ATOM    464  O   ASP A  67      18.694  54.767  92.414  1.00 18.91
ATOM    465  CB  ASP A  67      16.787  52.944  90.495  1.00 21.68
ATOM    466  CG  ASP A  67      16.824  51.580  89.801  1.00 25.22
ATOM    467  OD1 ASP A  67      17.340  50.629  90.370  1.00 23.32
ATOM    468  OD2 ASP A  67      16.349  51.434  88.666  1.00 26.83
ATOM    469  N   ALA A  68      16.485  55.059  92.773  1.00 16.48
ATOM    470  CA  ALA A  68      16.685  56.425  93.250  1.00 19.28
ATOM    471  C   ALA A  68      17.489  56.510  94.569  1.00 20.86
ATOM    472  O   ALA A  68      18.165  57.494  94.837  1.00 22.07
ATOM    473  CB  ALA A  68      15.330  57.134  93.419  1.00 19.81
ATOM    474  N   ASN A  69      17.472  55.371  95.299  1.00 23.11
ATOM    475  CA  ASN A  69      18.330  55.262  96.514  1.00 27.41
ATOM    476  C   ASN A  69      19.816  55.042  96.273  1.00 29.49
ATOM    477  O   ASN A  69      20.646  55.304  97.140  1.00 28.64
ATOM    478  CB  ASN A  69      17.933  54.145  97.466  1.00 24.19
ATOM    479  CG  ASN A  69      16.632  54.425  98.142  1.00 25.79
ATOM    480  OD1 ASN A  69      16.298  55.549  98.445  1.00 26.84
ATOM    481  ND2 ASN A  69      15.894  53.359  98.410  1.00 30.16
ATOM    482  N   LEU A  70      20.104  54.574  95.034  1.00 25.30
ATOM    483  CA  LEU A  70      21.514  54.442  94.627  1.00 23.20
ATOM    484  C   LEU A  70      22.329  55.691  94.640  1.00 21.41
ATOM    485  O   LEU A  70      22.013  56.696  94.028  1.00 23.50
ATOM    486  CB  LEU A  70      21.672  53.890  93.225  1.00 22.19
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ATOM    487  CG  LEU A  70      21.078  52.512  93.095  1.00 22.16
ATOM    488  CD1 LEU A  70      21.830  51.459  93.896  1.00 20.70
ATOM    489  CD2 LEU A  70      21.016  52.154  91.624  1.00 23.47
ATOM    490  N   SER A  71      23.450  55.563  95.304  1.00 21.73
ATOM    491  CA  SER A  71      24.527  56.515  95.119  1.00 22.25
ATOM    492  C   SER A  71      25.355  56.171  93.888  1.00 20.52
ATOM    493  O   SER A  71      25.269  55.081  93.357  1.00 23.70
ATOM    494  CB  SER A  71      25.453  56.521  96.349  1.00 22.74
ATOM    495  OG  SER A  71      26.232  55.303  96.432  1.00 28.68
ATOM    496  N   SER A  72      26.220  57.079  93.445  1.00 20.02
ATOM    497  CA  SER A  72      27.096  56.747  92.294  1.00 20.88
ATOM    498  C   SER A  72      27.860  55.479  92.410  1.00 22.52
ATOM    499  O   SER A  72      27.979  54.663  91.518  1.00 21.65
ATOM    500  CB  SER A  72      28.113  57.834  92.083  1.00 19.05
ATOM    501  OG  SER A  72      27.352  58.966  91.735  1.00 22.00
ATOM    502  N   GLY A  73      28.336  55.318  93.640  1.00 20.71
ATOM    503  CA  GLY A  73      28.979  54.068  94.006  1.00 16.81
ATOM    504  C   GLY A  73      28.146  52.783  93.939  1.00 15.97
ATOM    505  O   GLY A  73      28.697  51.705  93.753  1.00 20.02
ATOM    506  N   GLY A  74      26.818  52.915  94.046  1.00 16.07
ATOM    507  CA  GLY A  74      26.090  51.649  93.967  1.00 18.17
ATOM    508  C   GLY A  74      25.671  51.260  92.526  1.00 21.98
ATOM    509  O   GLY A  74      25.202  50.164  92.238  1.00 21.28
ATOM    510  N   VAL A  75      25.887  52.210  91.567  1.00 22.48
ATOM    511  CA  VAL A  75      25.521  51.777  90.174  1.00 22.71
ATOM    512  C   VAL A  75      26.174  50.493  89.628  1.00 18.50
ATOM    513  O   VAL A  75      25.497  49.573  89.210  1.00 20.32
ATOM    514  CB  VAL A  75      25.820  52.946  89.218  1.00 23.52
ATOM    515  CG1 VAL A  75      25.719  52.707  87.712  1.00 21.49
ATOM    516  CG2 VAL A  75      25.153  54.265  89.560  1.00 17.54
ATOM    517  N   ALA A  76      27.517  50.394  89.738  1.00 21.05
ATOM    518  CA  ALA A  76      28.149  49.125  89.372  1.00 20.51
ATOM    519  C   ALA A  76      27.414  47.875  89.826  1.00 24.64
ATOM    520  O   ALA A  76      27.033  47.028  89.015  1.00 24.90
ATOM    521  CB  ALA A  76      29.612  49.071  89.810  1.00 19.14
ATOM    522  N   ASN A  77      27.131  47.820  91.145  1.00 20.97
ATOM    523  CA  ASN A  77      26.463  46.601  91.622  1.00 17.62
ATOM    524  C   ASN A  77      25.019  46.464  91.205  1.00 16.57
ATOM    525  O   ASN A  77      24.536  45.350  91.024  1.00 19.24
ATOM    526  CB  ASN A  77      26.615  46.509  93.137  1.00 23.52
ATOM    527  CG  ASN A  77      25.817  45.362  93.677  1.00 21.57
ATOM    528  OD1 ASN A  77      24.672  45.508  94.079  1.00 26.66
ATOM    529  ND2 ASN A  77      26.435  44.202  93.627  1.00 24.64
ATOM    530  N   ALA A  78      24.377  47.638  91.017  1.00 17.23
ATOM    531  CA  ALA A  78      23.060  47.710  90.339  1.00 18.62
ATOM    532  C   ALA A  78      22.874  47.025  88.941  1.00 19.92
ATOM    533  O   ALA A  78      21.767  46.705  88.517  1.00 21.04
ATOM    534  CB  ALA A  78      22.636  49.160  90.208  1.00 13.62
ATOM    535  N   PHE A  79      24.025  46.748  88.292  1.00 19.22
ATOM    536  CA  PHE A  79      24.019  45.921  87.070  1.00 20.71
ATOM    537  C   PHE A  79      24.117  44.420  87.238  1.00 23.30
ATOM    538  O   PHE A  79      24.161  43.662  86.273  1.00 23.64
ATOM    539  CB  PHE A  79      25.116  46.352  86.082  1.00 18.00
ATOM    540  CG  PHE A  79      24.821  47.683  85.382  1.00 19.59
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ATOM    541  CD1 PHE A  79      25.181  48.903  85.984  1.00 17.05
ATOM    542  CD2 PHE A  79      24.214  47.687  84.104  1.00 18.56
ATOM    543  CE1 PHE A  79      25.026  50.119  85.298  1.00 19.06
ATOM    544  CE2 PHE A  79      24.040  48.906  83.419  1.00 15.97
ATOM    545  CZ  PHE A  79      24.500  50.104  83.989  1.00 17.87
ATOM    546  N   SER A  80      24.150  43.947  88.488  1.00 19.74
ATOM    547  CA  SER A  80      24.500  42.511  88.632  1.00 17.29
ATOM    548  C   SER A  80      23.601  41.501  87.944  1.00 18.57
ATOM    549  O   SER A  80      23.977  40.494  87.339  1.00 22.38
ATOM    550  CB  SER A  80      24.608  42.106  90.125  1.00 15.17
ATOM    551  OG  SER A  80      25.646  42.920  90.700  1.00 17.32
ATOM    552  N   GLY A  81      22.309  41.852  88.041  1.00 19.68
ATOM    553  CA  GLY A  81      21.271  41.005  87.413  1.00 22.50
ATOM    554  C   GLY A  81      21.293  40.977  85.855  1.00 24.85
ATOM    555  O   GLY A  81      21.318  39.939  85.211  1.00 23.46
ATOM    556  N   ALA A  82      21.380  42.197  85.279  1.00 24.60
ATOM    557  CA  ALA A  82      21.686  42.339  83.855  1.00 24.50
ATOM    558  C   ALA A  82      22.985  41.643  83.417  1.00 25.22
ATOM    559  O   ALA A  82      23.000  40.873  82.468  1.00 23.42
ATOM    560  CB  ALA A  82      21.649  43.819  83.470  1.00 20.35
ATOM    561  N   PHE A  83      24.050  41.874  84.197  1.00 23.76
ATOM    562  CA  PHE A  83      25.319  41.242  83.894  1.00 21.76
ATOM    563  C   PHE A  83      25.325  39.726  83.974  1.00 22.85
ATOM    564  O   PHE A  83      26.090  39.052  83.322  1.00 25.72
ATOM    565  CB  PHE A  83      26.349  41.867  84.792  1.00 20.01
ATOM    566  CG  PHE A  83      27.770  41.527  84.394  1.00 20.95
ATOM    567  CD1 PHE A  83      28.486  40.587  85.152  1.00 16.94
ATOM    568  CD2 PHE A  83      28.391  42.208  83.307  1.00 23.09
ATOM    569  CE1 PHE A  83      29.841  40.355  84.843  1.00 18.79
ATOM    570  CE2 PHE A  83      29.751  41.991  83.001  1.00 20.95
ATOM    571  CZ  PHE A  83      30.474  41.069  83.795  1.00 21.43
ATOM    572  N   GLY A  84      24.409  39.187  84.768  1.00 22.31
ATOM    573  CA  GLY A  84      24.478  37.740  84.865  1.00 24.39
ATOM    574  C   GLY A  84      25.199  37.163  86.093  1.00 29.49
ATOM    575  O   GLY A  84      25.158  35.963  86.362  1.00 32.27
ATOM    576  N   SER A  85      25.873  38.058  86.843  1.00 30.33
ATOM    577  CA  SER A  85      26.685  37.625  88.001  1.00 28.46
ATOM    578  C   SER A  85      27.047  38.788  88.936  1.00 29.06
ATOM    579  O   SER A  85      26.915  39.945  88.556  1.00 29.00
ATOM    580  CB  SER A  85      27.915  36.861  87.536  1.00 24.04
ATOM    581  OG  SER A  85      28.903  37.746  87.028  1.00 28.11
ATOM    582  N   PRO A  86      27.436  38.518  90.216  1.00 29.45
ATOM    583  CA  PRO A  86      27.599  39.650  91.122  1.00 26.27
ATOM    584  C   PRO A  86      28.721  40.513  90.733  1.00 22.75
ATOM    585  O   PRO A  86      29.830  40.064  90.530  1.00 22.43
ATOM    586  CB  PRO A  86      27.873  39.029  92.493  1.00 27.52
ATOM    587  CG  PRO A  86      27.284  37.627  92.399  1.00 27.61
ATOM    588  CD  PRO A  86      27.591  37.258  90.945  1.00 31.08
ATOM    589  N   ILE A  87      28.350  41.776  90.659  1.00 22.99
ATOM    590  CA  ILE A  87      29.363  42.816  90.469  1.00 24.80
ATOM    591  C   ILE A  87      29.642  43.494  91.811  1.00 25.76
ATOM    592  O   ILE A  87      28.956  44.424  92.220  1.00 24.68
ATOM    593  CB  ILE A  87      28.908  43.860  89.427  1.00 24.26
ATOM    594  CG1 ILE A  87      28.626  43.165  88.076  1.00 23.29
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ATOM    595  CG2 ILE A  87      29.997  44.923  89.306  1.00 23.62
ATOM    596  CD1 ILE A  87      27.925  44.100  87.092  1.00 21.95
ATOM    597  N   THR A  88      30.655  42.914  92.481  1.00 25.86
ATOM    598  CA  THR A  88      30.925  43.247  93.903  1.00 25.48
ATOM    599  C   THR A  88      32.418  43.249  94.166  1.00 25.25
ATOM    600  O   THR A  88      33.131  42.561  93.445  1.00 24.26
ATOM    601  CB  THR A  88      30.332  42.211  94.859  1.00 22.31
ATOM    602  OG1 THR A  88      31.102  41.029  94.702  1.00 25.42
ATOM    603  CG2 THR A  88      28.833  41.943  94.710  1.00 19.45
ATOM    604  N   GLU A  89      32.891  43.970  95.204  1.00 26.63
ATOM    605  CA  GLU A  89      34.322  43.845  95.577  1.00 25.29
ATOM    606  C   GLU A  89      34.810  42.429  95.889  1.00 25.30
ATOM    607  O   GLU A  89      35.924  41.999  95.611  1.00 26.30
ATOM    608  CB  GLU A  89      34.652  44.773  96.741  1.00 25.64
ATOM    609  CG  GLU A  89      34.334  46.193  96.340  1.00 26.52
ATOM    610  CD  GLU A  89      34.551  47.228  97.414  1.00 29.70
ATOM    611  OE1 GLU A  89      35.136  48.245  97.123  1.00 33.05
ATOM    612  OE2 GLU A  89      34.138  47.077  98.540  1.00 27.59
ATOM    613  N   LYS A  90      33.860  41.697  96.459  1.00 26.25
ATOM    614  CA  LYS A  90      34.095  40.310  96.883  1.00 28.81
ATOM    615  C   LYS A  90      34.285  39.313  95.780  1.00 28.19
ATOM    616  O   LYS A  90      35.206  38.518  95.773  1.00 30.49
ATOM    617  CB  LYS A  90      32.889  39.869  97.672  1.00 31.00
ATOM    618  CG  LYS A  90      32.956  38.478  98.228  1.00 37.00
ATOM    619  CD  LYS A  90      31.536  38.026  98.583  1.00 43.53
ATOM    620  CE  LYS A  90      31.386  36.504  98.712  1.00 50.17
ATOM    621  NZ  LYS A  90      32.257  35.875  97.701  1.00 60.80
ATOM    622  N   ASP A  91      33.324  39.416  94.870  1.00 28.68
ATOM    623  CA  ASP A  91      33.271  38.504  93.738  1.00 29.34
ATOM    624  C   ASP A  91      33.911  38.947  92.420  1.00 29.37
ATOM    625  O   ASP A  91      34.429  38.173  91.635  1.00 31.54
ATOM    626  CB  ASP A  91      31.827  38.162  93.438  1.00 30.91
ATOM    627  CG  ASP A  91      31.087  37.722  94.674  1.00 31.69
ATOM    628  OD1 ASP A  91      31.395  36.657  95.212  1.00 35.66
ATOM    629  OD2 ASP A  91      30.186  38.438  95.088  1.00 29.63
ATOM    630  N   ALA A  92      33.830  40.240  92.165  1.00 28.62
ATOM    631  CA  ALA A  92      34.443  40.713  90.919  1.00 26.75
ATOM    632  C   ALA A  92      35.255  41.981  91.131  1.00 26.94
ATOM    633  O   ALA A  92      34.937  43.081  90.690  1.00 26.94
ATOM    634  CB  ALA A  92      33.390  40.934  89.817  1.00 23.93
ATOM    635  N   PRO A  93      36.336  41.826  91.930  1.00 27.16
ATOM    636  CA  PRO A  93      37.151  43.015  92.274  1.00 25.78
ATOM    637  C   PRO A  93      37.832  43.865  91.160  1.00 24.62
ATOM    638  O   PRO A  93      37.844  45.098  91.178  1.00 22.75
ATOM    639  CB  PRO A  93      38.120  42.411  93.292  1.00 25.31
ATOM    640  CG  PRO A  93      38.219  40.926  92.945  1.00 23.54
ATOM    641  CD  PRO A  93      36.817  40.578  92.534  1.00 25.29
ATOM    642  N   ALA A  94      38.409  43.174  90.170  1.00 25.16
ATOM    643  CA  ALA A  94      38.954  43.886  89.005  1.00 23.77
ATOM    644  C   ALA A  94      37.923  44.715  88.249  1.00 17.72
ATOM    645  O   ALA A  94      38.116  45.897  88.005  1.00 19.91
ATOM    646  CB  ALA A  94      39.676  42.931  88.058  1.00 21.52
ATOM    647  N   LEU A  95      36.787  44.081  88.026  1.00 19.68
ATOM    648  CA  LEU A  95      35.577  44.770  87.539  1.00 20.23
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ATOM    649  C   LEU A  95      35.001  45.902  88.385  1.00 21.85
ATOM    650  O   LEU A  95      34.809  47.026  87.943  1.00 21.66
ATOM    651  CB  LEU A  95      34.466  43.755  87.271  1.00 20.70
ATOM    652  CG  LEU A  95      33.250  44.285  86.467  1.00 21.32
ATOM    653  CD1 LEU A  95      32.299  43.149  86.063  1.00 20.10
ATOM    654  CD2 LEU A  95      33.698  45.111  85.240  1.00 20.93
ATOM    655  N   HIS A  96      34.755  45.606  89.667  1.00 21.70
ATOM    656  CA  HIS A  96      34.313  46.691  90.543  1.00 17.84
ATOM    657  C   HIS A  96      35.214  47.924  90.580  1.00 17.76
ATOM    658  O   HIS A  96      34.767  49.069  90.496  1.00 19.44
ATOM    659  CB  HIS A  96      34.042  46.116  91.937  1.00 20.62
ATOM    660  CG  HIS A  96      32.934  46.893  92.613  1.00 21.96
ATOM    661  ND1 HIS A  96      33.021  48.094  93.229  1.00 21.34
ATOM    662  CD2 HIS A  96      31.614  46.470  92.680  1.00 24.62
ATOM    663  CE1 HIS A  96      31.790  48.442  93.690  1.00 18.16
ATOM    664  NE2 HIS A  96      30.923  47.437  93.338  1.00 23.59
ATOM    665  N   LYS A  97      36.539  47.639  90.629  1.00 18.11
ATOM    666  CA  LYS A  97      37.544  48.713  90.581  1.00 18.50
ATOM    667  C   LYS A  97      37.519  49.564  89.317  1.00 20.80
ATOM    668  O   LYS A  97      37.452  50.781  89.373  1.00 21.08
ATOM    669  CB  LYS A  97      38.924  48.085  90.766  1.00 17.26
ATOM    670  CG  LYS A  97      40.125  49.014  90.594  1.00 21.24
ATOM    671  CD  LYS A  97      40.283  50.213  91.525  1.00 30.10
ATOM    672  CE  LYS A  97      41.482  51.160  91.164  1.00 33.55
ATOM    673  NZ  LYS A  97      41.557  52.449  91.915  1.00 29.55
ATOM    674  N   LEU A  98      37.532  48.857  88.155  1.00 22.36
ATOM    675  CA  LEU A  98      37.291  49.555  86.851  1.00 22.04
ATOM    676  C   LEU A  98      36.128  50.581  86.806  1.00 17.59
ATOM    677  O   LEU A  98      36.223  51.763  86.522  1.00 18.13
ATOM    678  CB  LEU A  98      37.025  48.477  85.780  1.00 21.44
ATOM    679  CG  LEU A  98      36.766  49.042  84.375  1.00 20.93
ATOM    680  CD1 LEU A  98      36.265  47.902  83.493  1.00 22.92
ATOM    681  CD2 LEU A  98      37.963  49.801  83.811  1.00 18.27
ATOM    682  N   LEU A  99      34.977  50.024  87.188  1.00 19.16
ATOM    683  CA  LEU A  99      33.753  50.802  87.186  1.00 18.37
ATOM    684  C   LEU A  99      33.644  51.930  88.169  1.00 20.61
ATOM    685  O   LEU A  99      33.068  52.964  87.883  1.00 18.31
ATOM    686  CB  LEU A  99      32.545  49.874  87.263  1.00 18.90
ATOM    687  CG  LEU A  99      32.428  48.860  86.191  1.00 20.87
ATOM    688  CD1 LEU A  99      32.464  49.503  84.841  1.00 14.73
ATOM    689  CD2 LEU A  99      31.190  48.099  86.497  1.00 19.82
ATOM    690  N   THR A 100      34.252  51.692  89.359  1.00 22.03
ATOM    691  CA  THR A 100      34.357  52.777  90.360  1.00 19.41
ATOM    692  C   THR A 100      35.259  53.938  89.957  1.00 16.72
ATOM    693  O   THR A 100      34.984  55.118  90.136  1.00 16.55
ATOM    694  CB  THR A 100      34.889  52.174  91.674  1.00 19.50
ATOM    695  OG1 THR A 100      34.030  51.113  92.091  1.00 19.99
ATOM    696  CG2 THR A 100      35.092  53.221  92.758  1.00 21.12
ATOM    697  N   ASN A 101      36.397  53.537  89.358  1.00 20.56
ATOM    698  CA  ASN A 101      37.415  54.515  88.998  1.00 19.91
ATOM    699  C   ASN A 101      37.022  55.479  87.873  1.00 21.88
ATOM    700  O   ASN A 101      37.610  56.546  87.711  1.00 22.38
ATOM    701  CB  ASN A 101      38.692  53.763  88.716  1.00 20.98
ATOM    702  CG  ASN A 101      39.950  54.556  89.041  1.00 22.36
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ATOM    703  OD1 ASN A 101      40.938  53.978  89.459  1.00 30.43
ATOM    704  ND2 ASN A 101      39.964  55.868  88.889  1.00 23.45
ATOM    705  N   MET A 102      35.952  55.090  87.154  1.00 21.94
ATOM    706  CA  MET A 102      35.407  55.984  86.103  1.00 22.28
ATOM    707  C   MET A 102      34.142  56.755  86.455  1.00 22.36
ATOM    708  O   MET A 102      33.571  57.482  85.638  1.00 23.37
ATOM    709  CB  MET A 102      35.162  55.213  84.781  1.00 19.03
ATOM    710  CG  MET A 102      34.239  54.001  84.972  1.00 18.05
ATOM    711  SD  MET A 102      33.744  53.082  83.481  1.00 20.07
ATOM    712  CE  MET A 102      32.429  54.165  83.010  1.00 16.04
ATOM    713  N   ILE A 103      33.681  56.555  87.724  1.00 21.00
ATOM    714  CA  ILE A 103      32.441  57.221  88.180  1.00 18.78
ATOM    715  C   ILE A 103      32.371  58.697  87.833  1.00 17.38
ATOM    716  O   ILE A 103      31.413  59.152  87.245  1.00 18.08
ATOM    717  CB  ILE A 103      32.174  57.025  89.732  1.00 16.18
ATOM    718  CG1 ILE A 103      31.696  55.603  90.031  1.00 19.21
ATOM    719  CG2 ILE A 103      31.135  58.037  90.272  1.00 12.63
ATOM    720  CD1 ILE A 103      31.708  55.185  91.522  1.00 17.58
ATOM    721  N   GLU A 104      33.426  59.429  88.218  1.00 18.81
ATOM    722  CA  GLU A 104      33.369  60.900  88.092  1.00 18.36
ATOM    723  C   GLU A 104      33.828  61.520  86.772  1.00 19.60
ATOM    724  O   GLU A 104      33.420  62.606  86.365  1.00 19.13
ATOM    725  CB  GLU A 104      34.092  61.600  89.241  1.00 18.56
ATOM    726  CG  GLU A 104      33.446  61.448  90.617  1.00 19.21
ATOM    727  CD  GLU A 104      31.994  61.944  90.665  1.00 23.36
ATOM    728  OE1 GLU A 104      31.225  61.359  91.382  1.00 26.94
ATOM    729  OE2 GLU A 104      31.574  62.888  90.013  1.00 28.46
ATOM    730  N   ASP A 105      34.606  60.713  86.049  1.00 18.95
ATOM    731  CA  ASP A 105      34.743  60.936  84.587  1.00 17.40
ATOM    732  C   ASP A 105      33.378  61.099  83.886  1.00 15.12
ATOM    733  O   ASP A 105      33.104  62.102  83.234  1.00 18.61
ATOM    734  CB  ASP A 105      35.429  59.743  83.951  1.00 16.81
ATOM    735  CG  ASP A 105      36.831  59.545  84.440  1.00 15.10
ATOM    736  OD1 ASP A 105      37.573  60.520  84.573  1.00 19.01
ATOM    737  OD2 ASP A 105      37.177  58.402  84.685  1.00 15.56
ATOM    738  N   ALA A 106      32.500  60.091  84.096  1.00 15.05
ATOM    739  CA  ALA A 106      31.111  60.157  83.607  1.00 15.61
ATOM    740  C   ALA A 106      30.166  61.126  84.315  1.00 19.62
ATOM    741  O   ALA A 106      29.409  61.881  83.720  1.00 18.83
ATOM    742  CB  ALA A 106      30.467  58.782  83.682  1.00 11.73
ATOM    743  N   GLY A 107      30.263  61.102  85.674  1.00 21.49
ATOM    744  CA  GLY A 107      29.323  61.899  86.503  1.00 16.83
ATOM    745  C   GLY A 107      29.599  63.356  86.594  1.00 14.80
ATOM    746  O   GLY A 107      28.714  64.204  86.575  1.00 17.67
ATOM    747  N   ASP A 108      30.899  63.611  86.662  1.00 16.37
ATOM    748  CA  ASP A 108      31.305  65.002  86.772  1.00 17.18
ATOM    749  C   ASP A 108      31.877  65.572  85.485  1.00 18.56
ATOM    750  O   ASP A 108      31.324  66.472  84.877  1.00 18.77
ATOM    751  CB  ASP A 108      32.282  65.144  87.947  1.00 16.92
ATOM    752  CG  ASP A 108      32.862  66.530  88.143  1.00 21.34
ATOM    753  OD1 ASP A 108      32.247  67.528  87.812  1.00 23.69
ATOM    754  OD2 ASP A 108      33.983  66.642  88.614  1.00 27.32
ATOM    755  N   LEU A 109      33.049  65.065  85.107  1.00 18.48
ATOM    756  CA  LEU A 109      33.814  65.703  84.005  1.00 20.77
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ATOM    757  C   LEU A 109      32.954  65.922  82.752  1.00 21.46
ATOM    758  O   LEU A 109      32.905  67.026  82.199  1.00 23.24
ATOM    759  CB  LEU A 109      35.024  64.826  83.679  1.00 20.60
ATOM    760  CG  LEU A 109      36.393  65.285  84.113  1.00 21.65
ATOM    761  CD1 LEU A 109      37.408  64.213  84.096  1.00 17.20
ATOM    762  CD2 LEU A 109      36.469  66.186  85.254  1.00 20.25
ATOM    763  N   ALA A 110      32.206  64.812  82.422  1.00 21.81
ATOM    764  CA  ALA A 110      31.336  64.771  81.215  1.00 20.79
ATOM    765  C   ALA A 110      30.074  65.608  81.232  1.00 23.23
ATOM    766  O   ALA A 110      29.504  65.885  80.182  1.00 23.66
ATOM    767  CB  ALA A 110      30.921  63.333  80.868  1.00 20.49
ATOM    768  N   THR A 111      29.672  66.038  82.465  1.00 20.15
ATOM    769  CA  THR A 111      28.453  66.863  82.653  1.00 19.21
ATOM    770  C   THR A 111      28.654  68.292  83.132  1.00 18.58
ATOM    771  O   THR A 111      27.754  69.106  83.131  1.00 18.77
ATOM    772  CB  THR A 111      27.469  66.257  83.628  1.00 18.46
ATOM    773  OG1 THR A 111      28.011  66.399  84.949  1.00 23.25
ATOM    774  CG2 THR A 111      27.094  64.802  83.347  1.00 15.78
ATOM    775  N   ARG A 112      29.870  68.595  83.547  1.00 20.94
ATOM    776  CA  ARG A 112      30.068  69.805  84.369  1.00 22.62
ATOM    777  C   ARG A 112      29.745  71.185  83.786  1.00 23.68
ATOM    778  O   ARG A 112      29.035  72.025  84.325  1.00 21.09
ATOM    779  CB  ARG A 112      31.512  69.782  84.911  1.00 22.88
ATOM    780  CG  ARG A 112      31.847  70.852  85.952  1.00 22.67
ATOM    781  CD  ARG A 112      33.319  70.922  86.319  1.00 18.55
ATOM    782  NE  ARG A 112      33.831  69.709  86.930  1.00 22.11
ATOM    783  CZ  ARG A 112      35.138  69.496  86.853  1.00 21.99
ATOM    784  NH1 ARG A 112      35.949  70.322  86.227  1.00 23.29
ATOM    785  NH2 ARG A 112      35.623  68.436  87.414  1.00 23.83
ATOM    786  N   SER A 113      30.323  71.398  82.583  1.00 21.66
ATOM    787  CA  SER A 113      30.146  72.736  81.981  1.00 19.14
ATOM    788  C   SER A 113      28.721  73.124  81.629  1.00 20.09
ATOM    789  O   SER A 113      28.288  74.263  81.806  1.00 23.06
ATOM    790  CB  SER A 113      31.029  72.919  80.732  1.00 24.64
ATOM    791  OG  SER A 113      30.812  71.854  79.778  1.00 25.18
ATOM    792  N   ALA A 114      27.955  72.094  81.186  1.00 19.43
ATOM    793  CA  ALA A 114      26.510  72.272  80.944  1.00 17.15
ATOM    794  C   ALA A 114      25.695  72.377  82.247  1.00 16.95
ATOM    795  O   ALA A 114      24.890  73.293  82.402  1.00 17.79
ATOM    796  CB  ALA A 114      25.935  71.096  80.117  1.00 15.05
ATOM    797  N   LYS A 115      25.993  71.462  83.200  1.00 18.76
ATOM    798  CA  LYS A 115      25.431  71.618  84.559  1.00 20.52
ATOM    799  C   LYS A 115      25.524  73.029  85.143  1.00 18.24
ATOM    800  O   LYS A 115      24.535  73.710  85.429  1.00 19.61
ATOM    801  CB  LYS A 115      26.048  70.606  85.508  1.00 17.41
ATOM    802  CG  LYS A 115      25.304  69.294  85.482  1.00 22.93
ATOM    803  CD  LYS A 115      25.867  68.477  86.654  1.00 26.73
ATOM    804  CE  LYS A 115      25.353  67.039  86.850  1.00 26.14
ATOM    805  NZ  LYS A 115      23.888  67.023  87.009  1.00 26.36
ATOM    806  N   ASP A 116      26.784  73.454  85.203  1.00 19.25
ATOM    807  CA  ASP A 116      27.073  74.739  85.832  1.00 20.02
ATOM    808  C   ASP A 116      26.589  75.938  85.076  1.00 23.51
ATOM    809  O   ASP A 116      26.208  76.967  85.612  1.00 24.69
ATOM    810  CB  ASP A 116      28.573  74.896  86.053  1.00 22.26
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ATOM    811  CG  ASP A 116      29.203  73.871  87.029  1.00 25.80
ATOM    812  OD1 ASP A 116      28.502  73.085  87.657  1.00 27.77
ATOM    813  OD2 ASP A 116      30.431  73.847  87.166  1.00 28.44
ATOM    814  N   HIS A 117      26.596  75.793  83.741  1.00 23.53
ATOM    815  CA  HIS A 117      26.022  76.894  82.957  1.00 21.71
ATOM    816  C   HIS A 117      24.496  77.044  83.026  1.00 20.75
ATOM    817  O   HIS A 117      23.932  78.108  83.223  1.00 20.51
ATOM    818  CB  HIS A 117      26.536  76.787  81.501  1.00 22.93
ATOM    819  CG  HIS A 117      25.987  77.909  80.635  1.00 22.08
ATOM    820  ND1 HIS A 117      26.531  79.128  80.459  1.00 23.33
ATOM    821  CD2 HIS A 117      24.824  77.852  79.881  1.00 22.30
ATOM    822  CE1 HIS A 117      25.741  79.836  79.615  1.00 23.49
ATOM    823  NE2 HIS A 117      24.693  79.041  79.262  1.00 23.57
ATOM    824  N   TYR A 118      23.825  75.906  82.825  1.00 19.95
ATOM    825  CA  TYR A 118      22.363  76.013  82.795  1.00 19.91
ATOM    826  C   TYR A 118      21.711  76.075  84.159  1.00 22.89
ATOM    827  O   TYR A 118      20.615  76.596  84.278  1.00 22.53
ATOM    828  CB  TYR A 118      21.702  74.869  82.020  1.00 18.70
ATOM    829  CG  TYR A 118      22.112  74.964  80.550  1.00 19.86
ATOM    830  CD1 TYR A 118      21.604  76.030  79.775  1.00 20.81
ATOM    831  CD2 TYR A 118      22.998  74.004  80.005  1.00 20.19
ATOM    832  CE1 TYR A 118      21.964  76.104  78.410  1.00 24.18
ATOM    833  CE2 TYR A 118      23.393  74.097  78.652  1.00 22.07
ATOM    834  CZ  TYR A 118      22.841  75.133  77.869  1.00 24.85
ATOM    835  OH  TYR A 118      23.138  75.231  76.525  1.00 25.02
ATOM    836  N   MET A 119      22.385  75.453  85.158  1.00 22.72
ATOM    837  CA  MET A 119      21.795  75.408  86.516  1.00 25.49
ATOM    838  C   MET A 119      20.328  75.033  86.619  1.00 22.79
ATOM    839  O   MET A 119      19.526  75.639  87.309  1.00 23.65
ATOM    840  CB  MET A 119      22.009  76.758  87.200  1.00 31.90
ATOM    841  CG  MET A 119      23.479  77.200  87.296  1.00 41.79
ATOM    842  SD  MET A 119      23.683  78.779  88.163  1.00 50.25
ATOM    843  CE  MET A 119      22.932  79.838  86.910  1.00 48.37
ATOM    844  N   ARG A 120      19.958  74.021  85.840  1.00 21.05
ATOM    845  CA  ARG A 120      18.529  73.782  85.704  1.00 19.45
ATOM    846  C   ARG A 120      17.877  73.247  86.989  1.00 16.34
ATOM    847  O   ARG A 120      18.483  72.369  87.587  1.00 17.21
ATOM    848  CB  ARG A 120      18.345  72.757  84.558  1.00 16.25
ATOM    849  CG  ARG A 120      16.913  72.517  84.063  1.00 17.39
ATOM    850  CD  ARG A 120      16.775  71.558  82.842  1.00 19.83
ATOM    851  NE  ARG A 120      15.450  71.636  82.189  1.00 20.39
ATOM    852  CZ  ARG A 120      14.929  70.642  81.479  1.00 12.14
ATOM    853  NH1 ARG A 120      15.600  69.574  81.259  1.00 12.74
ATOM    854  NH2 ARG A 120      13.724  70.767  81.007  1.00 15.75
ATOM    855  N   ILE A 121      16.676  73.723  87.290  1.00 16.46
ATOM    856  CA  ILE A 121      15.807  73.186  88.360  1.00 22.91
ATOM    857  C   ILE A 121      15.258  71.795  88.080  1.00 23.50
ATOM    858  O   ILE A 121      14.648  71.571  87.043  1.00 26.14
ATOM    859  CB  ILE A 121      14.594  74.134  88.686  1.00 25.98
ATOM    860  CG1 ILE A 121      14.986  75.592  88.783  1.00 29.16
ATOM    861  CG2 ILE A 121      13.793  73.870  89.982  1.00 23.20
ATOM    862  CD1 ILE A 121      16.075  75.914  89.785  1.00 29.73
ATOM    863  N   ARG A 122      15.463  70.877  89.036  1.00 21.01
ATOM    864  CA  ARG A 122      14.883  69.534  88.976  1.00 19.80
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ATOM	865	C	ARG	A	122	13.372	69.532	89.091	1.00 17.61
ATOM	866	O	ARG	A	122	12.805	70.488	89.613	1.00 18.79
ATOM	867	CB	ARG	A	122	15.533	68.653	90.033	1.00 17.47
ATOM	868	CG	ARG	A	122	17.023	68.706	89.841	1.00 18.78
ATOM	869	CD	ARG	A	122	17.721	67.861	90.852	1.00 21.00
ATOM	870	NE	ARG	A	122	19.141	68.121	90.748	1.00 28.80
ATOM	871	CZ	ARG	A	122	19.914	67.081	90.491	1.00 34.31
ATOM	872	NH1	ARG	A	122	19.401	65.871	90.426	1.00 40.28
ATOM	873	NH2	ARG	A	122	21.196	67.257	90.289	1.00 32.47
ATOM	874	N	PRO	A	123	12.700	68.460	88.577	1.00 17.64
ATOM	875	CA	PRO	A	123	11.243	68.461	88.684	1.00 18.56
ATOM	876	C	PRO	A	123	10.668	68.630	90.118	1.00 20.86
ATOM	877	O	PRO	A	123	9.881	69.536	90.331	1.00 20.65
ATOM	878	CB	PRO	A	123	10.808	67.150	88.004	1.00 16.86
ATOM	879	CG	PRO	A	123	12.028	66.572	87.296	1.00 15.55
ATOM	880	CD	PRO	A	123	13.235	67.291	87.881	1.00 15.48
ATOM	881	N	PHE	A	124	11.077	67.771	91.105	1.00 21.82
ATOM	882	CA	PHE	A	124	10.489	67.948	92.468	1.00 20.01
ATOM	883	C	PHE	A	124	10.541	69.394	93.016	1.00 16.73
ATOM	884	O	PHE	A	124	9.581	69.970	93.486	1.00 17.82
ATOM	885	CB	PHE	A	124	11.044	66.869	93.422	1.00 17.84
ATOM	886	CG	PHE	A	124	12.484	67.199	93.795	1.00 19.87
ATOM	887	CD1	PHE	A	124	12.748	68.117	94.850	1.00 20.96
ATOM	888	CD2	PHE	A	124	13.554	66.632	93.075	1.00 19.87
ATOM	889	CE1	PHE	A	124	14.068	68.524	95.134	1.00 21.78
ATOM	890	CE2	PHE	A	124	14.881	67.014	93.381	1.00 21.98
ATOM	891	CZ	PHE	A	124	15.129	67.975	94.386	1.00 23.27
ATOM	892	N	ALA	A	125	11.681	70.039	92.775	1.00 18.30
ATOM	893	CA	ALA	A	125	11.866	71.464	93.089	1.00 20.06
ATOM	894	C	ALA	A	125	11.033	72.481	92.291	1.00 24.90
ATOM	895	O	ALA	A	125	10.455	73.445	92.789	1.00 24.77
ATOM	896	CB	ALA	A	125	13.358	71.840	92.990	1.00 16.96
ATOM	897	N	PHE	A	126	10.941	72.202	90.977	1.00 23.91
ATOM	898	CA	PHE	A	126	10.017	72.958	90.145	1.00 22.66
ATOM	899	C	PHE	A	126	8.590	72.919	90.692	1.00 20.74
ATOM	900	O	PHE	A	126	7.910	73.945	90.785	1.00 21.66
ATOM	901	CB	PHE	A	126	10.051	72.379	88.705	1.00 19.61
ATOM	902	CG	PHE	A	126	9.147	73.140	87.765	1.00 16.99
ATOM	903	CD1	PHE	A	126	9.669	74.211	87.022	1.00 15.15
ATOM	904	CD2	PHE	A	126	7.794	72.757	87.656	1.00 17.75
ATOM	905	CE1	PHE	A	126	8.824	74.913	86.144	1.00 14.16
ATOM	906	CE2	PHE	A	126	6.940	73.472	86.799	1.00 17.56
ATOM	907	CZ	PHE	A	126	7.471	74.538	86.048	1.00 12.93
ATOM	908	N	TYR	A	127	8.183	71.664	91.002	1.00 20.13
ATOM	909	CA	TYR	A	127	6.843	71.414	91.525	1.00 20.41
ATOM	910	C	TYR	A	127	6.642	71.689	93.032	1.00 24.41
ATOM	911	O	TYR	A	127	5.525	71.665	93.532	1.00 23.70
ATOM	912	CB	TYR	A	127	6.370	69.994	91.207	1.00 20.85
ATOM	913	CG	TYR	A	127	6.198	69.850	89.697	1.00 25.51
ATOM	914	CD1	TYR	A	127	7.200	69.185	88.947	1.00 27.02
ATOM	915	CD2	TYR	A	127	5.064	70.407	89.065	1.00 25.24
ATOM	916	CE1	TYR	A	127	7.057	69.037	87.552	1.00 28.75
ATOM	917	CE2	TYR	A	127	4.907	70.261	87.663	1.00 28.24
ATOM	918	CZ	TYR	A	127	5.891	69.543	86.936	1.00 28.76

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ATOM    919  OH  TYR A 127       5.716  69.314  85.588  1.00 27.53
ATOM    920  N   GLY A 128       7.764  71.960  93.723  1.00 23.74
ATOM    921  CA  GLY A 128       7.675  72.217  95.170  1.00 24.88
ATOM    922  C   GLY A 128       7.138  71.011  95.924  1.00 24.57
ATOM    923  O   GLY A 128       6.383  71.095  96.866  1.00 29.82
ATOM    924  N   VAL A 129       7.527  69.854  95.426  1.00 24.16
ATOM    925  CA  VAL A 129       7.144  68.604  96.044  1.00 23.21
ATOM    926  C   VAL A 129       8.377  67.802  96.358  1.00 24.99
ATOM    927  O   VAL A 129       9.529  68.188  96.189  1.00 25.90
ATOM    928  CB  VAL A 129       6.193  67.776  95.187  1.00 22.38
ATOM    929  CG1 VAL A 129       6.738  67.390  93.815  1.00 17.75
ATOM    930  CG2 VAL A 129       4.895  68.549  95.125  1.00 24.52
ATOM    931  N   SER A 130       8.089  66.617  96.832  1.00 25.98
ATOM    932  CA  SER A 130       9.242  65.724  96.973  1.00 29.71
ATOM    933  C   SER A 130       9.322  64.672  95.895  1.00 29.73
ATOM    934  O   SER A 130       8.403  64.485  95.103  1.00 30.28
ATOM    935  CB  SER A 130       9.183  64.999  98.309  1.00 35.36
ATOM    936  OG  SER A 130       7.964  64.244  98.427  1.00 41.61
ATOM    937  N   THR A 131      10.440  63.952  95.934  1.00 30.35
ATOM    938  CA  THR A 131      10.533  62.819  94.996  1.00 27.90
ATOM    939  C   THR A 131       9.733  61.569  95.361  1.00 28.80
ATOM    940  O   THR A 131       9.051  61.508  96.366  1.00 30.16
ATOM    941  CB  THR A 131      11.996  62.471  94.783  1.00 26.62
ATOM    942  OG1 THR A 131      12.500  61.841  95.953  1.00 29.21
ATOM    943  CG2 THR A 131      12.839  63.682  94.446  1.00 19.32
ATOM    944  N   CYS A 132       9.835  60.528  94.551  1.00 27.12
ATOM    945  CA  CYS A 132       9.203  59.271  94.996  1.00 27.75
ATOM    946  C   CYS A 132       9.911  58.540  96.137  1.00 28.96
ATOM    947  O   CYS A 132       9.556  57.487  96.634  1.00 28.80
ATOM    948  CB  CYS A 132       9.081  58.274  93.831  1.00 24.68
ATOM    949  SG  CYS A 132      10.538  57.273  93.459  1.00 24.12
ATOM    950  N   ASN A 133      11.021  59.158  96.447  1.00 33.31
ATOM    951  CA  ASN A 133      12.012  58.492  97.236  1.00 39.44
ATOM    952  C   ASN A 133      12.008  58.776  98.750  1.00 45.19
ATOM    953  O   ASN A 133      12.269  57.928  99.583  1.00 50.38
ATOM    954  CB  ASN A 133      13.285  58.876  96.522  1.00 35.83
ATOM    955  CG  ASN A 133      14.294  57.823  96.731  1.00 37.18
ATOM    956  OD1 ASN A 133      15.478  58.059  96.663  1.00 38.83
ATOM    957  ND2 ASN A 133      13.815  56.623  97.017  1.00 42.51
ATOM    958  N   THR A 134      11.642  59.993  99.118  1.00 48.69
ATOM    959  CA  THR A 134      12.585  61.110  98.925  1.00 56.17
ATOM    960  C   THR A 134      13.935  61.171  99.735  1.00 61.49
ATOM    961  OCT1 THR A 134      14.052  60.565 100.816  1.00 66.36
ATOM    962  OCT2 THR A 134      14.937  61.779  99.291  1.00 63.18
ATOM    963  CB  THR A 134      11.704  62.374  98.968  1.00 56.04
ATOM    964  OG1 THR A 134      12.306  63.614  98.469  1.00 56.23
ATOM    965  CG2 THR A 134      10.869  62.400 100.243  1.00 53.77
ATOM    966  N   GLN A 137      16.953  60.437 100.819  1.00100.00
ATOM    967  CA  GLN A 137      17.845  60.498 102.027  1.00 99.78
ATOM    968  C   GLN A 137      19.036  61.512 102.211  1.00 98.61
ATOM    969  O   GLN A 137      19.386  61.891 103.324  1.00 97.54
ATOM    970  CB  GLN A 137      18.343  59.083 102.397  1.00100.00
ATOM    971  CG  GLN A 137      17.669  58.407 103.615  1.00 99.38
ATOM    972  CD  GLN A 137      18.060  59.017 104.963  1.00 98.64
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ATOM    973  OE1 GLN A 137      17.484  58.784 106.009  1.00 98.03
ATOM    974  NE2 GLN A 137      19.086  59.847 104.972  1.00100.00
ATOM    975  N   ASP A 138      19.627  61.949 101.081  1.00 97.91
ATOM    976  CA  ASP A 138      20.479  63.151 101.162  1.00 95.99
ATOM    977  C   ASP A 138      19.761  64.494 101.063  1.00 93.86
ATOM    978  O   ASP A 138      18.589  64.579 100.711  1.00 92.76
ATOM    979  CB  ASP A 138      21.585  63.102 100.115  1.00 97.46
ATOM    980  CG  ASP A 138      22.893  62.963 100.866  1.00100.00
ATOM    981  OD1 ASP A 138      23.371  61.842 101.028  1.00100.00
ATOM    982  OD2 ASP A 138      23.432  63.969 101.333  1.00100.00
ATOM    983  N   LYS A 139      20.494  65.564 101.380  1.00 92.69
ATOM    984  CA  LYS A 139      19.813  66.855 101.218  1.00 91.50
ATOM    985  C   LYS A 139      19.719  67.313  99.775  1.00 88.92
ATOM    986  O   LYS A 139      20.557  68.082  99.308  1.00 89.47
ATOM    987  CB  LYS A 139      20.464  67.987 102.010  1.00 94.21
ATOM    988  CG  LYS A 139      19.574  69.246 102.020  1.00 96.82
ATOM    989  CD  LYS A 139      20.362  70.569 102.123  1.00 99.43
ATOM    990  CE  LYS A 139      20.785  71.241 100.793  1.00100.00
ATOM    991  NZ  LYS A 139      21.686  70.402  99.978  1.00100.00
ATOM    992  N   LEU A 140      18.654  66.828  99.114  1.00 85.87
ATOM    993  CA  LEU A 140      18.426  67.018  97.660  1.00 79.60
ATOM    994  C   LEU A 140      18.772  68.392  97.043  1.00 75.62
ATOM    995  O   LEU A 140      18.242  69.442  97.416  1.00 75.74
ATOM    996  CB  LEU A 140      16.981  66.620  97.283  1.00 77.27
ATOM    997  CG  LEU A 140      16.640  65.142  97.462  1.00 74.31
ATOM    998  CD1 LEU A 140      17.569  64.263  96.645  1.00 73.21
ATOM    999  CD2 LEU A 140      15.178  64.838  97.167  1.00 73.91
ATOM   1000  N   SER A 141      19.713  68.357  96.067  1.00 70.08
ATOM   1001  CA  SER A 141      19.868  69.619  95.320  1.00 64.86
ATOM   1002  C   SER A 141      18.713  69.941  94.396  1.00 61.18
ATOM   1003  O   SER A 141      18.189  69.080  93.708  1.00 61.77
ATOM   1004  CB  SER A 141      21.127  69.682  94.461  1.00 64.49
ATOM   1005  OG  SER A 141      21.354  71.028  94.002  1.00 66.43
ATOM   1006  N   LYS A 142      18.349  71.235  94.379  1.00 57.81
ATOM   1007  CA  LYS A 142      17.238  71.661  93.493  1.00 54.35
ATOM   1008  C   LYS A 142      17.498  71.829  91.964  1.00 45.94
ATOM   1009  O   LYS A 142      16.615  72.091  91.202  1.00 40.89
ATOM   1010  CB  LYS A 142      16.631  72.953  94.061  1.00 57.69
ATOM   1011  CG  LYS A 142      17.518  74.222  93.959  1.00 62.63
ATOM   1012  CD  LYS A 142      16.625  75.420  93.545  1.00 68.07
ATOM   1013  CE  LYS A 142      17.200  76.856  93.475  1.00 71.28
ATOM   1014  NZ  LYS A 142      16.136  77.844  93.162  1.00 70.88
ATOM   1015  N   ASN A 143      18.772  71.719  91.611  1.00 45.99
ATOM   1016  CA  ASN A 143      19.527  72.392  90.538  1.00 44.71
ATOM   1017  C   ASN A 143      20.592  71.481  89.878  1.00 42.57
ATOM   1018  O   ASN A 143      20.794  70.322  90.283  1.00 40.17
ATOM   1019  CB  ASN A 143      20.343  73.547  91.124  1.00 50.34
ATOM   1020  CG  ASN A 143      19.624  74.818  90.876  1.00 55.19
ATOM   1021  OD1 ASN A 143      18.441  74.944  91.098  1.00 59.21
ATOM   1022  ND2 ASN A 143      20.366  75.786  90.389  1.00 59.88
ATOM   1023  N   GLY A 144      21.265  72.084  88.819  1.00 39.72
ATOM   1024  CA  GLY A 144      22.264  71.401  87.958  1.00 26.74
ATOM   1025  C   GLY A 144      21.691  70.105  87.407  1.00 21.44
ATOM   1026  O   GLY A 144      22.343  69.071  87.322  1.00 25.78
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ATOM   1027  N   SER A 145      20.380  70.181  87.105  1.00 19.15
ATOM   1028  CA  SER A 145      19.590  69.023  86.596  1.00 20.14
ATOM   1029  C   SER A 145      20.077  68.461  85.223  1.00 20.58
ATOM   1030  O   SER A 145      20.121  67.279  84.943  1.00 21.22
ATOM   1031  CB  SER A 145      18.171  69.499  86.338  1.00 18.89
ATOM   1032  OG  SER A 145      17.219  68.526  86.712  1.00 28.02
ATOM   1033  N   TYR A 146      20.531  69.419  84.399  1.00 22.70
ATOM   1034  CA  TYR A 146      20.867  69.170  82.968  1.00 19.51
ATOM   1035  C   TYR A 146      22.366  69.260  82.633  1.00 16.00
ATOM   1036  O   TYR A 146      22.930  70.339  82.736  1.00 17.85
ATOM   1037  CB  TYR A 146      20.097  70.216  82.114  1.00 18.79
ATOM   1038  CG  TYR A 146      20.211  69.933  80.600  1.00 20.29
ATOM   1039  CD1 TYR A 146      19.310  69.027  80.015  1.00 19.58
ATOM   1040  CD2 TYR A 146      21.205  70.582  79.824  1.00 20.42
ATOM   1041  CE1 TYR A 146      19.389  68.781  78.631  1.00 23.13
ATOM   1042  CE2 TYR A 146      21.287  70.337  78.424  1.00 21.68
ATOM   1043  CZ  TYR A 146      20.349  69.449  77.849  1.00 20.03
ATOM   1044  OH  TYR A 146      20.320  69.236  76.483  1.00 21.40
ATOM   1045  N   PRO A 147      22.994  68.144  82.187  1.00 16.52
ATOM   1046  CA  PRO A 147      22.385  66.793  82.188  1.00 17.87
ATOM   1047  C   PRO A 147      22.496  66.069  83.580  1.00 20.67
ATOM   1048  O   PRO A 147      23.198  66.531  84.480  1.00 22.35
ATOM   1049  CB  PRO A 147      23.250  66.107  81.122  1.00 15.36
ATOM   1050  CG  PRO A 147      24.649  66.716  81.297  1.00 15.06
ATOM   1051  CD  PRO A 147      24.356  68.168  81.630  1.00 16.28
ATOM   1052  N   SER A 148      21.827  64.908  83.662  1.00 18.73
ATOM   1053  CA  SER A 148      21.951  64.019  84.823  1.00 19.51
ATOM   1054  C   SER A 148      23.269  63.281  84.930  1.00 19.93
ATOM   1055  O   SER A 148      23.601  62.439  84.108  1.00 20.09
ATOM   1056  CB  SER A 148      20.828  62.997  84.807  1.00 18.55
ATOM   1057  OG  SER A 148      20.990  61.966  85.780  1.00 19.29
ATOM   1058  N   GLY A 149      24.036  63.618  85.972  1.00 18.26
ATOM   1059  CA  GLY A 149      25.284  62.888  86.231  1.00 16.00
ATOM   1060  C   GLY A 149      25.096  61.411  86.577  1.00 19.38
ATOM   1061  O   GLY A 149      25.791  60.549  86.044  1.00 21.04
ATOM   1062  N   HIS A 150      24.085  61.096  87.427  1.00 20.25
ATOM   1063  CA  HIS A 150      23.690  59.680  87.624  1.00 19.01
ATOM   1064  C   HIS A 150      23.381  58.861  86.330  1.00 19.93
ATOM   1065  O   HIS A 150      23.833  57.725  86.145  1.00 20.78
ATOM   1066  CB  HIS A 150      22.507  59.533  88.619  1.00 18.01
ATOM   1067  CG  HIS A 150      22.162  58.068  88.909  1.00 20.60
ATOM   1068  ND1 HIS A 150      22.864  57.235  89.730  1.00 23.41
ATOM   1069  CD2 HIS A 150      21.117  57.313  88.374  1.00 21.91
ATOM   1070  CE1 HIS A 150      22.287  55.996  89.732  1.00 22.83
ATOM   1071  NE2 HIS A 150      21.220  56.051  88.893  1.00 24.19
ATOM   1072  N   THR A 151      22.593  59.482  85.432  1.00 18.98
ATOM   1073  CA  THR A 151      22.325  58.814  84.132  1.00 17.17
ATOM   1074  C   THR A 151      23.548  58.652  83.228  1.00 13.61
ATOM   1075  O   THR A 151      23.814  57.594  82.659  1.00 17.00
ATOM   1076  CB  THR A 151      21.270  59.590  83.407  1.00 16.57
ATOM   1077  OG1 THR A 151      20.137  59.738  84.258  1.00 18.00
ATOM   1078  CG2 THR A 151      20.898  58.983  82.045  1.00 13.22
ATOM   1079  N   SER A 152      24.361  59.722  83.197  1.00 14.30
ATOM   1080  CA  SER A 152      25.687  59.598  82.557  1.00 15.99
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ATOM	1081	C	SER	A	152	26.575	58.446	83.029	1.00 19.72
ATOM	1082	O	SER	A	152	27.086	57.628	82.255	1.00 17.38
ATOM	1083	CB	SER	A	152	26.434	60.927	82.644	1.00 12.58
ATOM	1084	OG	SER	A	152	27.648	60.861	81.911	1.00 16.08
ATOM	1085	N	ILE	A	153	26.662	58.340	84.403	1.00 20.60
ATOM	1086	CA	ILE	A	153	27.272	57.121	85.017	1.00 16.15
ATOM	1087	C	ILE	A	153	26.622	55.802	84.631	1.00 10.76
ATOM	1088	O	ILE	A	153	27.293	54.850	84.262	1.00 14.38
ATOM	1089	CB	ILE	A	153	27.384	57.170	86.608	1.00 15.02
ATOM	1090	CG1	ILE	A	153	28.187	58.421	86.963	1.00 16.37
ATOM	1091	CG2	ILE	A	153	28.154	55.944	87.164	1.00 12.98
ATOM	1092	CD1	ILE	A	153	27.870	59.034	88.338	1.00 16.58
ATOM	1093	N	GLY	A	154	25.285	55.763	84.720	1.00 9.72
ATOM	1094	CA	GLY	A	154	24.662	54.476	84.397	1.00 13.63
ATOM	1095	C	GLY	A	154	24.843	54.033	82.910	1.00 16.00
ATOM	1096	O	GLY	A	154	25.022	52.866	82.571	1.00 16.37
ATOM	1097	N	TRP	A	155	24.801	55.047	82.025	1.00 16.77
ATOM	1098	CA	TRP	A	155	24.960	54.747	80.589	1.00 16.73
ATOM	1099	C	TRP	A	155	26.378	54.351	80.200	1.00 15.90
ATOM	1100	O	TRP	A	155	26.656	53.301	79.628	1.00 18.41
ATOM	1101	CB	TRP	A	155	24.442	55.940	79.771	1.00 16.52
ATOM	1102	CG	TRP	A	155	24.320	55.475	78.321	1.00 19.11
ATOM	1103	CD1	TRP	A	155	25.009	56.015	77.210	1.00 18.94
ATOM	1104	CD2	TRP	A	155	23.471	54.426	77.765	1.00 18.98
ATOM	1105	NE1	TRP	A	155	24.679	55.352	76.044	1.00 17.77
ATOM	1106	CE2	TRP	A	155	23.781	54.322	76.357	1.00 22.22
ATOM	1107	CE3	TRP	A	155	22.585	53.486	78.337	1.00 19.43
ATOM	1108	CZ2	TRP	A	155	23.084	53.377	75.564	1.00 17.99
ATOM	1109	CZ3	TRP	A	155	21.913	52.538	77.537	1.00 19.46
ATOM	1110	CH2	TRP	A	155	22.191	52.464	76.158	1.00 17.84
ATOM	1111	N	ALA	A	156	27.299	55.209	80.623	1.00 15.37
ATOM	1112	CA	ALA	A	156	28.702	54.836	80.515	1.00 14.36
ATOM	1113	C	ALA	A	156	29.156	53.503	81.108	1.00 19.46
ATOM	1114	O	ALA	A	156	29.895	52.723	80.528	1.00 19.86
ATOM	1115	CB	ALA	A	156	29.564	55.918	81.136	1.00 15.83
ATOM	1116	N	THR	A	157	28.651	53.207	82.327	1.00 19.95
ATOM	1117	CA	THR	A	157	28.820	51.832	82.831	1.00 17.52
ATOM	1118	C	THR	A	157	28.177	50.744	81.994	1.00 15.22
ATOM	1119	O	THR	A	157	28.825	49.745	81.765	1.00 19.09
ATOM	1120	CB	THR	A	157	28.328	51.667	84.291	1.00 14.92
ATOM	1121	OG1	THR	A	157	28.932	52.679	85.054	1.00 18.29
ATOM	1122	CG2	THR	A	157	28.620	50.327	84.944	1.00 13.21
ATOM	1123	N	ALA	A	158	26.930	50.947	81.535	1.00 14.63
ATOM	1124	CA	ALA	A	158	26.365	49.936	80.621	1.00 17.10
ATOM	1125	C	ALA	A	158	27.213	49.686	79.354	1.00 15.52
ATOM	1126	O	ALA	A	158	27.539	48.565	79.025	1.00 16.52
ATOM	1127	CB	ALA	A	158	24.942	50.300	80.203	1.00 13.99
ATOM	1128	N	LEU	A	159	27.655	50.766	78.705	1.00 17.91
ATOM	1129	CA	LEU	A	159	28.613	50.615	77.580	1.00 17.69
ATOM	1130	C	LEU	A	159	29.895	49.851	77.846	1.00 19.93
ATOM	1131	O	LEU	A	159	30.277	48.954	77.092	1.00 19.21
ATOM	1132	CB	LEU	A	159	28.959	51.971	76.939	1.00 14.08
ATOM	1133	CG	LEU	A	159	27.744	52.759	76.396	1.00 11.97
ATOM	1134	CD1	LEU	A	159	27.045	52.105	75.210	1.00 12.90

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ATOM   1135  CD2 LEU A 159      28.177  54.158  76.046  1.00 12.40
ATOM   1136  N   VAL A 160      30.547  50.181  79.014  1.00 21.70
ATOM   1137  CA  VAL A 160      31.713  49.315  79.310  1.00 20.85
ATOM   1138  C   VAL A 160      31.429  47.877  79.767  1.00 21.33
ATOM   1139  O   VAL A 160      32.086  46.918  79.409  1.00 20.74
ATOM   1140  CB  VAL A 160      32.574  50.032  80.367  1.00 21.59
ATOM   1141  CG1 VAL A 160      33.518  49.143  81.174  1.00 20.11
ATOM   1142  CG2 VAL A 160      33.299  51.307  79.977  1.00 20.10
ATOM   1143  N   LEU A 161      30.351  47.711  80.542  1.00 21.86
ATOM   1144  CA  LEU A 161      29.850  46.366  80.838  1.00 20.50
ATOM   1145  C   LEU A 161      29.462  45.490  79.613  1.00 23.16
ATOM   1146  O   LEU A 161      29.753  44.294  79.564  1.00 22.89
ATOM   1147  CB  LEU A 161      28.623  46.472  81.733  1.00 18.65
ATOM   1148  CG  LEU A 161      28.685  46.438  83.262  1.00 20.99
ATOM   1149  CD1 LEU A 161      27.473  46.378  84.194  1.00 21.43
ATOM   1150  CD2 LEU A 161      29.729  45.492  83.855  1.00 19.30
ATOM   1151  N   ALA A 162      28.755  46.146  78.648  1.00 21.20
ATOM   1152  CA  ALA A 162      28.384  45.496  77.361  1.00 19.14
ATOM   1153  C   ALA A 162      29.591  44.948  76.586  1.00 17.15
ATOM   1154  O   ALA A 162      29.620  43.812  76.133  1.00 20.85
ATOM   1155  CB  ALA A 162      27.581  46.464  76.500  1.00 17.38
ATOM   1156  N   GLU A 163      30.663  45.745  76.603  1.00 14.74
ATOM   1157  CA  GLU A 163      31.962  45.262  76.118  1.00 17.48
ATOM   1158  C   GLU A 163      32.648  44.080  76.824  1.00 22.07
ATOM   1159  O   GLU A 163      33.271  43.216  76.227  1.00 23.96
ATOM   1160  CB  GLU A 163      32.915  46.448  76.000  1.00 13.52
ATOM   1161  CG  GLU A 163      34.227  46.018  75.359  1.00 13.04
ATOM   1162  CD  GLU A 163      35.240  47.119  75.338  1.00 15.88
ATOM   1163  OE1 GLU A 163      36.427  46.814  75.269  1.00 19.78
ATOM   1164  OE2 GLU A 163      34.873  48.290  75.377  1.00 20.10
ATOM   1165  N   ILE A 164      32.504  44.039  78.153  1.00 20.17
ATOM   1166  CA  ILE A 164      32.996  42.869  78.905  1.00 18.72
ATOM   1167  C   ILE A 164      32.164  41.609  78.757  1.00 17.39
ATOM   1168  O   ILE A 164      32.635  40.481  78.674  1.00 20.44
ATOM   1169  CB  ILE A 164      33.132  43.293  80.382  1.00 20.67
ATOM   1170  CG1 ILE A 164      34.222  44.361  80.452  1.00 18.83
ATOM   1171  CG2 ILE A 164      33.398  42.110  81.345  1.00 20.70
ATOM   1172  CD1 ILE A 164      34.144  45.084  81.793  1.00 21.31
ATOM   1173  N   ASN A 165      30.869  41.846  78.704  1.00 18.41
ATOM   1174  CA  ASN A 165      29.979  40.712  78.524  1.00 21.36
ATOM   1175  C   ASN A 165      28.957  40.867  77.375  1.00 23.74
ATOM   1176  O   ASN A 165      27.753  40.988  77.563  1.00 23.14
ATOM   1177  CB  ASN A 165      29.324  40.404  79.878  1.00 21.69
ATOM   1178  CG  ASN A 165      28.471  39.156  79.861  1.00 25.72
ATOM   1179  OD1 ASN A 165      28.469  38.307  78.967  1.00 29.37
ATOM   1180  ND2 ASN A 165      27.730  39.051  80.951  1.00 27.39
ATOM   1181  N   PRO A 166      29.466  40.814  76.118  1.00 25.93
ATOM   1182  CA  PRO A 166      28.556  40.971  74.957  1.00 26.27
ATOM   1183  C   PRO A 166      27.447  39.924  74.837  1.00 24.06
ATOM   1184  O   PRO A 166      26.361  40.184  74.360  1.00 24.11
ATOM   1185  CB  PRO A 166      29.517  40.940  73.781  1.00 25.15
ATOM   1186  CG  PRO A 166      30.731  40.254  74.255  1.00 27.68
ATOM   1187  CD  PRO A 166      30.849  40.613  75.728  1.00 24.89
ATOM   1188  N   GLN A 167      27.679  38.741  75.385  1.00 25.15
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ATOM   1189  CA  GLN A 167      26.552  37.804  75.508  1.00 27.09
ATOM   1190  C   GLN A 167      25.275  38.321  76.189  1.00 25.06
ATOM   1191  O   GLN A 167      24.162  37.932  75.883  1.00 23.75
ATOM   1192  CB  GLN A 167      27.080  36.617  76.282  1.00 35.50
ATOM   1193  CG  GLN A 167      27.386  35.380  75.468  1.00 52.12
ATOM   1194  CD  GLN A 167      26.074  34.747  75.000  1.00 65.78
ATOM   1195  OE1 GLN A 167      25.950  34.245  73.889  1.00 72.02
ATOM   1196  NE2 GLN A 167      25.047  34.780  75.867  1.00 71.50
ATOM   1197  N   ARG A 168      25.513  39.242  77.156  1.00 26.04
ATOM   1198  CA  ARG A 168      24.389  39.900  77.837  1.00 25.63
ATOM   1199  C   ARG A 168      24.242  41.368  77.558  1.00 23.88
ATOM   1200  O   ARG A 168      23.632  42.106  78.308  1.00 24.09
ATOM   1201  CB  ARG A 168      24.452  39.638  79.337  1.00 24.63
ATOM   1202  CG  ARG A 168      24.087  38.171  79.408  1.00 25.72
ATOM   1203  CD  ARG A 168      23.986  37.617  80.802  1.00 33.72
ATOM   1204  NE  ARG A 168      22.970  38.302  81.587  1.00 35.38
ATOM   1205  CZ  ARG A 168      21.729  37.877  81.680  1.00 35.91
ATOM   1206  NH1 ARG A 168      20.908  38.482  82.490  1.00 34.99
ATOM   1207  NH2 ARG A 168      21.314  36.867  80.975  1.00 39.28
ATOM   1208  N   GLN A 169      24.838  41.810  76.424  1.00 23.22
ATOM   1209  CA  GLN A 169      24.820  43.228  76.080  1.00 19.99
ATOM   1210  C   GLN A 169      23.441  43.850  76.067  1.00 21.38
ATOM   1211  O   GLN A 169      23.216  44.969  76.492  1.00 24.60
ATOM   1212  CB  GLN A 169      25.571  43.510  74.769  1.00 22.07
ATOM   1213  CG  GLN A 169      24.970  42.878  73.494  1.00 20.93
ATOM   1214  CD  GLN A 169      25.716  43.287  72.207  1.00 23.42
ATOM   1215  OE1 GLN A 169      26.680  44.055  72.202  1.00 24.82
ATOM   1216  NE2 GLN A 169      25.186  42.738  71.109  1.00 13.86
ATOM   1217  N   ASN A 170      22.455  43.089  75.600  1.00 20.48
ATOM   1218  CA  ASN A 170      21.138  43.736  75.525  1.00 20.97
ATOM   1219  C   ASN A 170      20.484  44.007  76.892  1.00 19.73
ATOM   1220  O   ASN A 170      19.852  45.020  77.128  1.00 18.90
ATOM   1221  CB  ASN A 170      20.195  42.930  74.595  1.00 24.43
ATOM   1222  CG  ASN A 170      20.763  42.881  73.153  1.00 25.82
ATOM   1223  OD1 ASN A 170      20.842  43.862  72.440  1.00 26.22
ATOM   1224  ND2 ASN A 170      21.197  41.709  72.734  1.00 25.47
ATOM   1225  N   GLU A 171      20.680  43.042  77.790  1.00 21.27
ATOM   1226  CA  GLU A 171      20.166  43.146  79.157  1.00 19.84
ATOM   1227  C   GLU A 171      20.849  44.220  79.926  1.00 16.23
ATOM   1228  O   GLU A 171      20.199  45.026  80.575  1.00 20.87
ATOM   1229  CB  GLU A 171      20.317  41.822  79.892  1.00 20.47
ATOM   1230  CG  GLU A 171      19.412  40.750  79.312  1.00 24.15
ATOM   1231  CD  GLU A 171      20.157  39.825  78.376  1.00 26.37
ATOM   1232  OE1 GLU A 171      21.076  40.256  77.685  1.00 23.54
ATOM   1233  OE2 GLU A 171      19.801  38.645  78.363  1.00 32.14
ATOM   1234  N   ILE A 172      22.169  44.236  79.751  1.00 17.53
ATOM   1235  CA  ILE A 172      23.029  45.318  80.257  1.00 16.90
ATOM   1236  C   ILE A 172      22.679  46.733  79.813  1.00 20.21
ATOM   1237  O   ILE A 172      22.454  47.636  80.617  1.00 18.91
ATOM   1238  CB  ILE A 172      24.507  44.992  79.956  1.00 17.00
ATOM   1239  CG1 ILE A 172      25.000  43.685  80.613  1.00 15.78
ATOM   1240  CG2 ILE A 172      25.426  46.163  80.300  1.00 18.13
ATOM   1241  CD1 ILE A 172      26.426  43.320  80.163  1.00 13.83
ATOM   1242  N   LEU A 173      22.575  46.909  78.468  1.00 20.75
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ATOM   1243  CA  LEU A 173      22.107  48.206  77.946  1.00 17.70
ATOM   1244  C   LEU A 173      20.699  48.622  78.396  1.00 16.26
ATOM   1245  O   LEU A 173      20.376  49.774  78.663  1.00 18.18
ATOM   1246  CB  LEU A 173      22.176  48.201  76.419  1.00 16.81
ATOM   1247  CG  LEU A 173      23.600  48.021  75.966  1.00 18.73
ATOM   1248  CD1 LEU A 173      24.410  49.282  76.138  1.00 16.97
ATOM   1249  CD2 LEU A 173      23.619  47.550  74.505  1.00 23.91
ATOM   1250  N   LYS A 174      19.835  47.625  78.475  1.00 15.86
ATOM   1251  CA  LYS A 174      18.494  47.970  78.945  1.00 19.21
ATOM   1252  C   LYS A 174      18.453  48.415  80.429  1.00 21.86
ATOM   1253  O   LYS A 174      17.800  49.404  80.737  1.00 20.00
ATOM   1254  CB  LYS A 174      17.577  46.773  78.700  1.00 20.19
ATOM   1255  CG  LYS A 174      16.094  47.130  78.731  1.00 27.84
ATOM   1256  CD  LYS A 174      15.799  48.368  77.858  1.00 36.14
ATOM   1257  CE  LYS A 174      14.309  48.628  77.774  1.00 40.56
ATOM   1258  NZ  LYS A 174      13.775  48.622  79.156  1.00 49.45
ATOM   1259  N   ARG A 175      19.250  47.698  81.286  1.00 21.93
ATOM   1260  CA  ARG A 175      19.476  48.145  82.686  1.00 19.65
ATOM   1261  C   ARG A 175      20.037  49.561  82.807  1.00 16.70
ATOM   1262  O   ARG A 175      19.476  50.419  83.468  1.00 18.12
ATOM   1263  CB  ARG A 175      20.345  47.126  83.467  1.00 21.25
ATOM   1264  CG  ARG A 175      20.608  47.477  84.954  1.00 22.00
ATOM   1265  CD  ARG A 175      19.259  47.669  85.656  1.00 25.63
ATOM   1266  NE  ARG A 175      19.435  47.923  87.095  1.00 29.77
ATOM   1267  CZ  ARG A 175      18.601  48.672  87.834  1.00 27.58
ATOM   1268  NH1 ARG A 175      17.550  49.301  87.355  1.00 24.74
ATOM   1269  NH2 ARG A 175      18.792  48.780  89.073  1.00 27.45
ATOM   1270  N   GLY A 176      21.122  49.800  82.048  1.00 17.53
ATOM   1271  CA  GLY A 176      21.698  51.143  81.893  1.00 17.18
ATOM   1272  C   GLY A 176      20.679  52.214  81.552  1.00 21.18
ATOM   1273  O   GLY A 176      20.582  53.278  82.149  1.00 21.85
ATOM   1274  N   TYR A 177      19.847  51.887  80.547  1.00 22.48
ATOM   1275  CA  TYR A 177      18.756  52.787  80.117  1.00 20.51
ATOM   1276  C   TYR A 177      17.754  53.106  81.242  1.00 18.92
ATOM   1277  O   TYR A 177      17.406  54.246  81.542  1.00 15.84
ATOM   1278  CB  TYR A 177      18.007  52.120  78.939  1.00 23.76
ATOM   1279  CG  TYR A 177      17.210  53.131  78.177  1.00 24.47
ATOM   1280  CD1 TYR A 177      15.817  53.306  78.397  1.00 26.61
ATOM   1281  CD2 TYR A 177      17.941  53.869  77.236  1.00 29.40
ATOM   1282  CE1 TYR A 177      15.139  54.308  77.661  1.00 29.08
ATOM   1283  CE2 TYR A 177      17.270  54.855  76.515  1.00 30.70
ATOM   1284  CZ  TYR A 177      15.899  55.092  76.747  1.00 32.01
ATOM   1285  OH  TYR A 177      15.401  56.167  76.020  1.00 41.52
ATOM   1286  N   GLU A 178      17.354  52.008  81.867  1.00 19.45
ATOM   1287  CA  GLU A 178      16.429  52.094  82.972  1.00 22.02
ATOM   1288  C   GLU A 178      16.820  52.802  84.236  1.00 20.09
ATOM   1289  O   GLU A 178      16.001  53.492  84.805  1.00 21.41
ATOM   1290  CB  GLU A 178      16.010  50.731  83.357  1.00 25.45
ATOM   1291  CG  GLU A 178      15.173  50.032  82.303  1.00 34.73
ATOM   1292  CD  GLU A 178      13.893  50.810  81.951  1.00 40.36
ATOM   1293  OE1 GLU A 178      13.432  51.667  82.707  1.00 33.51
ATOM   1294  OE2 GLU A 178      13.352  50.556  80.876  1.00 45.77
ATOM   1295  N   LEU A 179      18.090  52.695  84.609  1.00 19.63
ATOM   1296  CA  LEU A 179      18.655  53.567  85.665  1.00 19.10
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ATOM	1297	C	LEU	A	179	18.366	55.051	85.511	1.00 20.25
ATOM	1298	O	LEU	A	179	17.838	55.726	86.374	1.00 19.26
ATOM	1299	CB	LEU	A	179	20.178	53.361	85.813	1.00 17.45
ATOM	1300	CG	LEU	A	179	20.610	52.013	86.426	1.00 18.23
ATOM	1301	CD1	LEU	A	179	20.093	51.829	87.873	1.00 16.98
ATOM	1302	CD2	LEU	A	179	22.124	51.811	86.340	1.00 16.34
ATOM	1303	N	GLY	A	180	18.647	55.545	84.290	1.00 19.84
ATOM	1304	CA	GLY	A	180	18.171	56.903	83.987	1.00 15.90
ATOM	1305	C	GLY	A	180	16.655	57.072	84.027	1.00 17.05
ATOM	1306	O	GLY	A	180	16.130	58.031	84.564	1.00 19.49
ATOM	1307	N	GLN	A	181	15.923	56.068	83.478	1.00 18.19
ATOM	1308	CA	GLN	A	181	14.448	56.207	83.522	1.00 17.92
ATOM	1309	C	GLN	A	181	13.822	56.265	84.938	1.00 18.20
ATOM	1310	O	GLN	A	181	12.903	57.008	85.258	1.00 19.66
ATOM	1311	CB	GLN	A	181	13.716	55.189	82.620	1.00 15.39
ATOM	1312	CG	GLN	A	181	13.936	55.324	81.073	1.00 15.18
ATOM	1313	CD	GLN	A	181	13.823	56.763	80.634	1.00 13.30
ATOM	1314	OE1	GLN	A	181	14.769	57.400	80.176	1.00 17.23
ATOM	1315	NE2	GLN	A	181	12.626	57.300	80.812	1.00 12.82
ATOM	1316	N	SER	A	182	14.440	55.497	85.821	1.00 20.03
ATOM	1317	CA	SER	A	182	14.156	55.600	87.273	1.00 16.56
ATOM	1318	C	SER	A	182	14.209	56.973	87.871	1.00 14.78
ATOM	1319	O	SER	A	182	13.305	57.369	88.582	1.00 19.49
ATOM	1320	CB	SER	A	182	15.056	54.630	88.021	1.00 15.31
ATOM	1321	OG	SER	A	182	14.563	53.332	87.714	1.00 16.70
ATOM	1322	N	ARG	A	183	15.246	57.738	87.523	1.00 16.99
ATOM	1323	CA	ARG	A	183	15.289	59.169	87.858	1.00 15.54
ATOM	1324	C	ARG	A	183	14.161	60.085	87.309	1.00 19.55
ATOM	1325	O	ARG	A	183	13.693	61.039	87.956	1.00 17.04
ATOM	1326	CB	ARG	A	183	16.661	59.785	87.569	1.00 14.08
ATOM	1327	CG	ARG	A	183	17.811	59.024	88.265	1.00 19.49
ATOM	1328	CD	ARG	A	183	17.716	59.007	89.816	1.00 21.46
ATOM	1329	NE	ARG	A	183	18.519	57.930	90.434	1.00 22.16
ATOM	1330	CZ	ARG	A	183	19.509	58.121	91.300	1.00 19.15
ATOM	1331	NH1	ARG	A	183	19.913	59.335	91.541	1.00 16.50
ATOM	1332	NH2	ARG	A	183	20.042	57.081	91.914	1.00 18.34
ATOM	1333	N	VAL	A	184	13.681	59.704	86.076	1.00 21.38
ATOM	1334	CA	VAL	A	184	12.494	60.417	85.589	1.00 19.02
ATOM	1335	C	VAL	A	184	11.194	60.051	86.301	1.00 16.98
ATOM	1336	O	VAL	A	184	10.468	60.913	86.800	1.00 18.45
ATOM	1337	CB	VAL	A	184	12.368	60.194	84.070	1.00 19.28
ATOM	1338	CG1	VAL	A	184	11.057	60.717	83.486	1.00 17.84
ATOM	1339	CG2	VAL	A	184	13.517	60.605	83.169	1.00 15.69
ATOM	1340	N	ILE	A	185	10.977	58.734	86.408	1.00 17.32
ATOM	1341	CA	ILE	A	185	9.834	58.241	87.202	1.00 21.14
ATOM	1342	C	ILE	A	185	9.790	58.797	88.672	1.00 22.77
ATOM	1343	O	ILE	A	185	8.749	59.230	89.142	1.00 22.95
ATOM	1344	CB	ILE	A	185	9.810	56.695	87.193	1.00 20.65
ATOM	1345	CG1	ILE	A	185	9.555	56.195	85.758	1.00 18.43
ATOM	1346	CG2	ILE	A	185	8.776	56.133	88.202	1.00 18.29
ATOM	1347	CD1	ILE	A	185	9.914	54.734	85.494	1.00 14.60
ATOM	1348	N	CYS	A	186	10.976	58.837	89.332	1.00 20.34
ATOM	1349	CA	CYS	A	186	11.005	59.262	90.745	1.00 21.26
ATOM	1350	C	CYS	A	186	10.979	60.766	90.931	1.00 22.80

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ATOM	1351	O	CYS	A	186	10.775	61.304	92.009	1.00 23.98
ATOM	1352	CB	CYS	A	186	12.220	58.669	91.429	1.00 21.60
ATOM	1353	SG	CYS	A	186	12.075	58.576	93.237	1.00 25.16
ATOM	1354	N	GLY	A	187	11.149	61.494	89.814	1.00 20.34
ATOM	1355	CA	GLY	A	187	11.023	62.949	89.981	1.00 13.75
ATOM	1356	C	GLY	A	187	12.351	63.676	90.197	1.00 14.55
ATOM	1357	O	GLY	A	187	12.436	64.871	90.453	1.00 16.08
ATOM	1358	N	TYR	A	188	13.434	62.894	90.072	1.00 14.86
ATOM	1359	CA	TYR	A	188	14.736	63.526	90.226	1.00 16.85
ATOM	1360	C	TYR	A	188	15.214	64.366	89.046	1.00 20.24
ATOM	1361	O	TYR	A	188	15.979	65.318	89.192	1.00 18.42
ATOM	1362	CB	TYR	A	188	15.758	62.441	90.479	1.00 21.64
ATOM	1363	CG	TYR	A	188	15.683	61.913	91.899	1.00 27.20
ATOM	1364	CD1	TYR	A	188	16.748	62.226	92.737	1.00 32.00
ATOM	1365	CD2	TYR	A	188	14.604	61.128	92.365	1.00 30.81
ATOM	1366	CE1	TYR	A	188	16.727	61.784	94.071	1.00 35.38
ATOM	1367	CE2	TYR	A	188	14.570	60.695	93.705	1.00 30.65
ATOM	1368	CZ	TYR	A	188	15.624	61.071	94.556	1.00 35.88
ATOM	1369	OH	TYR	A	188	15.607	60.787	95.912	1.00 41.39
ATOM	1370	N	HIS	A	189	14.751	63.890	87.862	1.00 19.35
ATOM	1371	CA	HIS	A	189	15.215	64.475	86.589	1.00 19.83
ATOM	1372	C	HIS	A	189	14.108	64.550	85.551	1.00 16.85
ATOM	1373	O	HIS	A	189	13.232	63.689	85.533	1.00 18.10
ATOM	1374	CB	HIS	A	189	16.360	63.648	86.032	1.00 15.43
ATOM	1375	CG	HIS	A	189	17.677	64.142	86.589	1.00 16.05
ATOM	1376	ND1	HIS	A	189	18.154	65.384	86.414	1.00 17.24
ATOM	1377	CD2	HIS	A	189	18.581	63.433	87.382	1.00 17.58
ATOM	1378	CE1	HIS	A	189	19.347	65.503	87.080	1.00 16.38
ATOM	1379	NE2	HIS	A	189	19.587	64.304	87.667	1.00 18.68
ATOM	1380	N	TRP	A	190	14.183	65.611	84.723	1.00 18.99
ATOM	1381	CA	TRP	A	190	13.341	65.719	83.490	1.00 17.22
ATOM	1382	C	TRP	A	190	13.768	64.690	82.453	1.00 15.79
ATOM	1383	O	TRP	A	190	14.942	64.354	82.416	1.00 15.77
ATOM	1384	CB	TRP	A	190	13.502	67.102	82.855	1.00 16.12
ATOM	1385	CG	TRP	A	190	13.134	68.178	83.857	1.00 12.39
ATOM	1386	CD1	TRP	A	190	14.033	69.073	84.454	1.00 10.88
ATOM	1387	CD2	TRP	A	190	11.800	68.581	84.295	1.00 14.35
ATOM	1388	NE1	TRP	A	190	13.343	69.989	85.186	1.00 13.77
ATOM	1389	CE2	TRP	A	190	11.976	69.731	85.141	1.00 10.71
ATOM	1390	CE3	TRP	A	190	10.505	68.036	84.094	1.00 14.68
ATOM	1391	CZ2	TRP	A	190	10.845	70.401	85.666	1.00 12.89
ATOM	1392	CZ3	TRP	A	190	9.393	68.700	84.672	1.00 16.93
ATOM	1393	CH2	TRP	A	190	9.557	69.875	85.441	1.00 12.82
ATOM	1394	N	GLN	A	191	12.859	64.188	81.613	1.00 16.92
ATOM	1395	CA	GLN	A	191	13.316	63.234	80.569	1.00 17.49
ATOM	1396	C	GLN	A	191	14.519	63.720	79.696	1.00 15.64
ATOM	1397	O	GLN	A	191	15.508	63.054	79.429	1.00 16.52
ATOM	1398	CB	GLN	A	191	12.113	62.829	79.721	1.00 15.28
ATOM	1399	CG	GLN	A	191	12.522	61.832	78.632	1.00 17.26
ATOM	1400	CD	GLN	A	191	12.860	60.493	79.206	1.00 16.84
ATOM	1401	OE1	GLN	A	191	12.086	59.920	79.946	1.00 21.21
ATOM	1402	NE2	GLN	A	191	14.027	59.971	78.864	1.00 16.69
ATOM	1403	N	SER	A	192	14.448	65.016	79.389	1.00 17.04
ATOM	1404	CA	SER	A	192	15.564	65.622	78.666	1.00 15.81

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ATOM   1405  C    SER A 192      16.899  65.683  79.339  1.00 17.61
ATOM   1406  O    SER A 192      17.937  65.640  78.700  1.00 16.65
ATOM   1407  CB   SER A 192      15.209  67.005  78.211  1.00 15.87
ATOM   1408  OG   SER A 192      14.862  67.833  79.313  1.00 21.12
ATOM   1409  N    ASP A 193      16.886  65.712  80.681  1.00 17.44
ATOM   1410  CA   ASP A 193      18.190  65.648  81.360  1.00 15.23
ATOM   1411  C    ASP A 193      18.889  64.316  81.197  1.00 12.26
ATOM   1412  O    ASP A 193      20.113  64.204  81.054  1.00 14.16
ATOM   1413  CB   ASP A 193      18.036  65.832  82.887  1.00 16.01
ATOM   1414  CG   ASP A 193      17.367  67.116  83.311  1.00 14.57
ATOM   1415  OD1  ASP A 193      17.503  68.180  82.691  1.00 15.89
ATOM   1416  OD2  ASP A 193      16.673  67.038  84.312  1.00 19.14
ATOM   1417  N    VAL A 194      18.025  63.283  81.216  1.00 11.48
ATOM   1418  CA   VAL A 194      18.425  61.882  81.108  1.00 13.18
ATOM   1419  C    VAL A 194      18.851  61.478  79.663  1.00 15.63
ATOM   1420  O    VAL A 194      19.852  60.822  79.387  1.00 15.79
ATOM   1421  CB   VAL A 194      17.210  61.135  81.625  1.00 16.33
ATOM   1422  CG1  VAL A 194      17.152  61.202  83.184  1.00 19.26
ATOM   1423  CG2  VAL A 194      17.079  59.712  81.106  1.00 17.70
ATOM   1424  N    ASP A 195      18.050  61.992  78.728  1.00 16.54
ATOM   1425  CA   ASP A 195      18.488  61.921  77.332  1.00 16.55
ATOM   1426  C    ASP A 195      19.801  62.636  77.029  1.00 14.74
ATOM   1427  O    ASP A 195      20.758  62.076  76.519  1.00 18.92
ATOM   1428  CB   ASP A 195      17.367  62.455  76.477  1.00 15.91
ATOM   1429  CG   ASP A 195      16.139  61.563  76.560  1.00 19.86
ATOM   1430  OD1  ASP A 195      16.153  60.385  76.922  1.00 27.62
ATOM   1431  OD2  ASP A 195      15.090  62.069  76.264  1.00 26.20
ATOM   1432  N    ALA A 196      19.902  63.900  77.450  1.00 15.85
ATOM   1433  CA   ALA A 196      21.203  64.555  77.312  1.00 14.73
ATOM   1434  C    ALA A 196      22.383  63.806  77.932  1.00 18.85
ATOM   1435  O    ALA A 196      23.512  63.751  77.429  1.00 21.06
ATOM   1436  CB   ALA A 196      21.134  65.950  77.904  1.00 13.59
ATOM   1437  N    ALA A 197      22.056  63.177  79.091  1.00 19.14
ATOM   1438  CA   ALA A 197      23.098  62.442  79.808  1.00 17.62
ATOM   1439  C    ALA A 197      23.644  61.202  79.090  1.00 17.71
ATOM   1440  O    ALA A 197      24.851  60.931  79.104  1.00 18.18
ATOM   1441  CB   ALA A 197      22.587  62.002  81.181  1.00 15.91
ATOM   1442  N    ARG A 198      22.711  60.477  78.418  1.00 16.04
ATOM   1443  CA   ARG A 198      23.238  59.409  77.565  1.00 15.41
ATOM   1444  C    ARG A 198      24.179  59.843  76.413  1.00 14.99
ATOM   1445  O    ARG A 198      25.194  59.219  76.113  1.00 17.07
ATOM   1446  CB   ARG A 198      22.136  58.469  77.080  1.00 14.50
ATOM   1447  CG   ARG A 198      21.195  58.043  78.179  1.00 16.67
ATOM   1448  CD   ARG A 198      20.142  57.044  77.730  1.00 19.20
ATOM   1449  NE   ARG A 198      19.280  56.629  78.849  1.00 22.72
ATOM   1450  CZ   ARG A 198      18.003  57.012  79.061  1.00 22.30
ATOM   1451  NH1  ARG A 198      17.412  57.905  78.325  1.00 20.95
ATOM   1452  NH2  ARG A 198      17.292  56.518  80.045  1.00 21.57
ATOM   1453  N    VAL A 199      23.907  61.030  75.842  1.00 17.69
ATOM   1454  CA   VAL A 199      24.961  61.510  74.913  1.00 17.44
ATOM   1455  C    VAL A 199      26.376  61.736  75.457  1.00 18.48
ATOM   1456  O    VAL A 199      27.360  61.145  74.988  1.00 19.25
ATOM   1457  CB   VAL A 199      24.452  62.820  74.284  1.00 15.93
ATOM   1458  CG1  VAL A 199      25.350  63.540  73.279  1.00 11.45
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ATOM   1459  CG2 VAL A 199      23.072  62.640  73.680  1.00 15.33
ATOM   1460  N   VAL A 200      26.471  62.548  76.550  1.00 18.52
ATOM   1461  CA  VAL A 200      27.822  62.723  77.153  1.00 16.27
ATOM   1462  C   VAL A 200      28.442  61.461  77.794  1.00 14.37
ATOM   1463  O   VAL A 200      29.643  61.247  77.797  1.00 17.25
ATOM   1464  CB  VAL A 200      27.811  63.938  78.100  1.00 16.56
ATOM   1465  CG1 VAL A 200      26.761  63.894  79.246  1.00 13.52
ATOM   1466  CG2 VAL A 200      27.666  65.177  77.226  1.00 17.14
ATOM   1467  N   GLY A 201      27.556  60.570  78.283  1.00 14.98
ATOM   1468  CA  GLY A 201      27.998  59.297  78.836  1.00 13.20
ATOM   1469  C   GLY A 201      28.609  58.377  77.824  1.00 16.81
ATOM   1470  O   GLY A 201      29.588  57.701  78.067  1.00 17.03
ATOM   1471  N   SER A 202      28.034  58.413  76.614  1.00 17.82
ATOM   1472  CA  SER A 202      28.757  57.750  75.509  1.00 16.70
ATOM   1473  C   SER A 202      30.087  58.390  75.104  1.00 13.91
ATOM   1474  O   SER A 202      31.117  57.765  74.939  1.00 18.47
ATOM   1475  CB  SER A 202      27.813  57.511  74.303  1.00 15.94
ATOM   1476  OG  SER A 202      27.634  58.737  73.600  1.00 19.26
ATOM   1477  N   ALA A 203      30.094  59.719  75.030  1.00 14.76
ATOM   1478  CA  ALA A 203      31.333  60.383  74.641  1.00 14.41
ATOM   1479  C   ALA A 203      32.527  60.110  75.563  1.00 17.92
ATOM   1480  O   ALA A 203      33.652  59.803  75.177  1.00 16.36
ATOM   1481  CB  ALA A 203      31.042  61.872  74.572  1.00 13.61
ATOM   1482  N   VAL A 204      32.200  60.126  76.880  1.00 18.27
ATOM   1483  CA  VAL A 204      33.290  59.835  77.823  1.00 16.45
ATOM   1484  C   VAL A 204      33.834  58.394  77.718  1.00 13.88
ATOM   1485  O   VAL A 204      35.015  58.177  77.916  1.00 17.64
ATOM   1486  CB  VAL A 204      32.893  60.245  79.285  1.00 16.63
ATOM   1487  CG1 VAL A 204      34.131  60.323  80.177  1.00 16.51
ATOM   1488  CG2 VAL A 204      31.836  59.301  79.862  1.00 13.53
ATOM   1489  N   VAL A 205      32.971  57.392  77.355  1.00 15.31
ATOM   1490  CA  VAL A 205      33.648  56.100  77.152  1.00 17.30
ATOM   1491  C   VAL A 205      34.636  56.018  75.953  1.00 19.20
ATOM   1492  O   VAL A 205      35.644  55.311  76.002  1.00 19.08
ATOM   1493  CB  VAL A 205      32.563  55.014  77.035  1.00 19.10
ATOM   1494  CG1 VAL A 205      32.812  53.549  76.681  1.00 16.51
ATOM   1495  CG2 VAL A 205      31.797  55.088  78.343  1.00 17.27
ATOM   1496  N   ALA A 206      34.409  56.893  74.934  1.00 21.03
ATOM   1497  CA  ALA A 206      35.452  57.018  73.888  1.00 19.17
ATOM   1498  C   ALA A 206      36.765  57.498  74.469  1.00 19.27
ATOM   1499  O   ALA A 206      37.809  56.868  74.353  1.00 18.59
ATOM   1500  CB  ALA A 206      34.982  57.988  72.809  1.00 16.60
ATOM   1501  N   THR A 207      36.643  58.597  75.234  1.00 20.20
ATOM   1502  CA  THR A 207      37.873  59.078  75.903  1.00 20.52
ATOM   1503  C   THR A 207      38.613  58.126  76.838  1.00 21.84
ATOM   1504  O   THR A 207      39.831  58.030  76.898  1.00 23.45
ATOM   1505  CB  THR A 207      37.659  60.341  76.674  1.00 19.84
ATOM   1506  OG1 THR A 207      36.577  61.100  76.137  1.00 20.11
ATOM   1507  CG2 THR A 207      38.945  61.141  76.710  1.00 21.15
ATOM   1508  N   LEU A 208      37.806  57.345  77.574  1.00 23.89
ATOM   1509  CA  LEU A 208      38.323  56.332  78.510  1.00 22.19
ATOM   1510  C   LEU A 208      39.165  55.285  77.783  1.00 25.28
ATOM   1511  O   LEU A 208      40.232  54.880  78.228  1.00 24.41
ATOM   1512  CB  LEU A 208      37.170  55.700  79.312  1.00 21.93
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ATOM   1513  CG  LEU A 208      36.238  56.370  80.325  1.00 24.99
ATOM   1514  CD1 LEU A 208      35.117  55.631  81.058  1.00 26.55
ATOM   1515  CD2 LEU A 208      37.303  56.685  81.377  1.00 22.67
ATOM   1516  N   HIS A 209      38.666  54.873  76.588  1.00 24.64
ATOM   1517  CA  HIS A 209      39.495  53.970  75.766  1.00 20.81
ATOM   1518  C   HIS A 209      40.863  54.530  75.277  1.00 19.15
ATOM   1519  O   HIS A 209      41.807  53.872  74.934  1.00 20.30
ATOM   1520  CB  HIS A 209      38.656  53.449  74.595  1.00 16.87
ATOM   1521  CG  HIS A 209      37.588  52.476  74.994  1.00 13.42
ATOM   1522  ND1 HIS A 209      36.335  52.782  75.375  1.00 14.12
ATOM   1523  CD2 HIS A 209      37.686  51.099  74.975  1.00 12.37
ATOM   1524  CE1 HIS A 209      35.653  51.616  75.586  1.00 10.36
ATOM   1525  NE2 HIS A 209      36.493  50.590  75.334  1.00 13.53
ATOM   1526  N   THR A 210      41.035  55.827  75.336  1.00 20.82
ATOM   1527  CA  THR A 210      42.393  56.384  75.116  1.00 21.40
ATOM   1528  C   THR A 210      43.396  56.387  76.298  1.00 26.34
ATOM   1529  O   THR A 210      44.567  56.745  76.188  1.00 27.83
ATOM   1530  CB  THR A 210      42.315  57.836  74.662  1.00 22.02
ATOM   1531  OG1 THR A 210      42.096  58.723  75.795  1.00 25.04
ATOM   1532  CG2 THR A 210      41.307  58.070  73.528  1.00 20.46
ATOM   1533  N   ASN A 211      42.844  56.032  77.482  1.00 26.32
ATOM   1534  CA  ASN A 211      43.544  56.135  78.782  1.00 23.64
ATOM   1535  C   ASN A 211      44.212  54.824  79.204  1.00 20.62
ATOM   1536  O   ASN A 211      43.591  53.785  79.374  1.00 22.28
ATOM   1537  CB  ASN A 211      42.563  56.749  79.827  1.00 24.99
ATOM   1538  CG  ASN A 211      43.226  56.863  81.206  1.00 24.09
ATOM   1539  OD1 ASN A 211      43.320  55.886  81.935  1.00 25.25
ATOM   1540  ND2 ASN A 211      43.689  58.038  81.566  1.00 21.24
ATOM   1541  N   PRO A 212      45.563  54.884  79.353  1.00 22.01
ATOM   1542  CA  PRO A 212      46.337  53.660  79.633  1.00 21.52
ATOM   1543  C   PRO A 212      45.859  52.883  80.848  1.00 22.52
ATOM   1544  O   PRO A 212      45.670  51.673  80.882  1.00 22.12
ATOM   1545  CB  PRO A 212      47.743  54.190  79.845  1.00 22.52
ATOM   1546  CG  PRO A 212      47.805  55.535  79.117  1.00 25.40
ATOM   1547  CD  PRO A 212      46.391  56.076  79.175  1.00 22.01
ATOM   1548  N   ALA A 213      45.626  53.674  81.897  1.00 23.96
ATOM   1549  CA  ALA A 213      45.139  53.025  83.140  1.00 23.57
ATOM   1550  C   ALA A 213      43.797  52.337  83.019  1.00 21.76
ATOM   1551  O   ALA A 213      43.600  51.185  83.403  1.00 24.96
ATOM   1552  CB  ALA A 213      45.039  54.071  84.259  1.00 21.55
ATOM   1553  N   PHE A 214      42.885  53.085  82.373  1.00 19.30
ATOM   1554  CA  PHE A 214      41.617  52.431  82.017  1.00 20.02
ATOM   1555  C   PHE A 214      41.798  51.170  81.197  1.00 20.38
ATOM   1556  O   PHE A 214      41.255  50.120  81.510  1.00 18.82
ATOM   1557  CB  PHE A 214      40.690  53.445  81.314  1.00 23.58
ATOM   1558  CG  PHE A 214      39.367  52.839  80.840  1.00 26.35
ATOM   1559  CD1 PHE A 214      38.249  52.765  81.711  1.00 24.66
ATOM   1560  CD2 PHE A 214      39.262  52.365  79.507  1.00 23.08
ATOM   1561  CE1 PHE A 214      37.032  52.191  81.265  1.00 27.05
ATOM   1562  CE2 PHE A 214      38.052  51.792  79.077  1.00 19.74
ATOM   1563  CZ  PHE A 214      36.951  51.697  79.944  1.00 22.04
ATOM   1564  N   GLN A 215      42.654  51.298  80.149  1.00 22.02
ATOM   1565  CA  GLN A 215      42.904  50.137  79.275  1.00 21.33
ATOM   1566  C   GLN A 215      43.354  48.889  79.995  1.00 21.73
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ATOM   1567  O   GLN A 215      42.823  47.783  79.875  1.00 21.17
ATOM   1568  CB  GLN A 215      43.970  50.520  78.278  1.00 22.37
ATOM   1569  CG  GLN A 215      43.483  51.517  77.261  1.00 22.30
ATOM   1570  CD  GLN A 215      44.662  52.026  76.479  1.00 28.18
ATOM   1571  OE1 GLN A 215      45.821  51.695  76.664  1.00 32.27
ATOM   1572  NE2 GLN A 215      44.359  52.947  75.605  1.00 28.35
ATOM   1573  N   GLN A 216      44.375  49.154  80.845  1.00 24.60
ATOM   1574  CA  GLN A 216      44.876  48.089  81.718  1.00 25.81
ATOM   1575  C   GLN A 216      43.909  47.530  82.715  1.00 23.07
ATOM   1576  O   GLN A 216      43.822  46.328  82.899  1.00 22.91
ATOM   1577  CB  GLN A 216      46.052  48.544  82.517  1.00 33.78
ATOM   1578  CG  GLN A 216      47.181  49.037  81.631  1.00 49.94
ATOM   1579  CD  GLN A 216      48.161  49.693  82.574  1.00 61.57
ATOM   1580  OE1 GLN A 216      48.354  49.243  83.704  1.00 69.34
ATOM   1581  NE2 GLN A 216      48.737  50.805  82.114  1.00 63.50
ATOM   1582  N   GLN A 217      43.155  48.439  83.377  1.00 22.58
ATOM   1583  CA  GLN A 217      42.099  47.917  84.261  1.00 23.69
ATOM   1584  C   GLN A 217      40.971  47.113  83.590  1.00 24.92
ATOM   1585  O   GLN A 217      40.480  46.102  84.088  1.00 24.09
ATOM   1586  CB  GLN A 217      41.565  49.042  85.189  1.00 23.44
ATOM   1587  CG  GLN A 217      40.720  48.541  86.407  1.00 23.24
ATOM   1588  CD  GLN A 217      41.489  47.589  87.335  1.00 21.58
ATOM   1589  OE1 GLN A 217      42.676  47.749  87.598  1.00 24.82
ATOM   1590  NE2 GLN A 217      40.827  46.516  87.744  1.00 19.85
ATOM   1591  N   LEU A 218      40.628  47.595  82.390  1.00 26.00
ATOM   1592  CA  LEU A 218      39.701  46.859  81.532  1.00 23.47
ATOM   1593  C   LEU A 218      40.195  45.495  81.093  1.00 21.96
ATOM   1594  O   LEU A 218      39.476  44.515  81.209  1.00 22.51
ATOM   1595  CB  LEU A 218      39.309  47.724  80.327  1.00 23.65
ATOM   1596  CG  LEU A 218      38.292  47.073  79.369  1.00 21.04
ATOM   1597  CD1 LEU A 218      38.103  47.980  78.168  1.00 25.30
ATOM   1598  CD2 LEU A 218      36.952  46.736  80.004  1.00 13.22
ATOM   1599  N   GLN A 219      41.451  45.425  80.640  1.00 23.49
ATOM   1600  CA  GLN A 219      42.033  44.079  80.457  1.00 29.37
ATOM   1601  C   GLN A 219      41.880  43.156  81.681  1.00 29.60
ATOM   1602  O   GLN A 219      41.455  42.016  81.569  1.00 29.63
ATOM   1603  CB  GLN A 219      43.544  44.131  80.199  1.00 37.46
ATOM   1604  CG  GLN A 219      44.052  44.703  78.867  1.00 51.74
ATOM   1605  CD  GLN A 219      45.511  45.267  78.911  1.00 60.29
ATOM   1606  OE1 GLN A 219      46.415  44.774  79.568  1.00 65.45
ATOM   1607  NE2 GLN A 219      45.764  46.352  78.161  1.00 60.81
ATOM   1608  N   LYS A 220      42.206  43.722  82.879  1.00 28.28
ATOM   1609  CA  LYS A 220      42.004  42.926  84.111  1.00 26.68
ATOM   1610  C   LYS A 220      40.588  42.446  84.386  1.00 24.54
ATOM   1611  O   LYS A 220      40.347  41.275  84.640  1.00 26.87
ATOM   1612  CB  LYS A 220      42.591  43.631  85.319  1.00 29.93
ATOM   1613  CG  LYS A 220      44.019  43.952  84.934  1.00 36.96
ATOM   1614  CD  LYS A 220      45.015  44.044  86.081  1.00 47.54
ATOM   1615  CE  LYS A 220      44.741  45.121  87.121  1.00 55.28
ATOM   1616  NZ  LYS A 220      44.868  46.456  86.510  1.00 61.92
ATOM   1617  N   ALA A 221      39.630  43.379  84.217  1.00 21.04
ATOM   1618  CA  ALA A 221      38.215  42.960  84.307  1.00 18.69
ATOM   1619  C   ALA A 221      37.761  41.903  83.291  1.00 24.31
ATOM   1620  O   ALA A 221      37.095  40.921  83.598  1.00 26.78
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ATOM   1621  CB  ALA A 221      37.306  44.177  84.140  1.00 14.85
ATOM   1622  N   LYS A 222      38.223  42.106  82.029  1.00 24.15
ATOM   1623  CA  LYS A 222      38.065  41.018  81.046  1.00 23.96
ATOM   1624  C   LYS A 222      38.668  39.675  81.431  1.00 22.61
ATOM   1625  O   LYS A 222      38.023  38.628  81.422  1.00 21.88
ATOM   1626  CB  LYS A 222      38.591  41.444  79.659  1.00 22.92
ATOM   1627  CG  LYS A 222      37.682  42.516  79.109  1.00 22.76
ATOM   1628  CD  LYS A 222      38.038  42.903  77.691  1.00 22.50
ATOM   1629  CE  LYS A 222      37.050  43.918  77.109  1.00 22.07
ATOM   1630  NZ  LYS A 222      37.556  44.613  75.909  1.00 21.47
ATOM   1631  N   ALA A 223      39.949  39.728  81.830  1.00 22.84
ATOM   1632  CA  ALA A 223      40.533  38.472  82.353  1.00 25.17
ATOM   1633  C   ALA A 223      39.812  37.842  83.543  1.00 26.19
ATOM   1634  O   ALA A 223      39.534  36.652  83.573  1.00 27.99
ATOM   1635  CB  ALA A 223      42.013  38.638  82.691  1.00 21.36
ATOM   1636  N   GLU A 224      39.424  38.696  84.487  1.00 27.72
ATOM   1637  CA  GLU A 224      38.643  38.187  85.610  1.00 27.59
ATOM   1638  C   GLU A 224      37.338  37.525  85.191  1.00 28.89
ATOM   1639  O   GLU A 224      36.971  36.412  85.568  1.00 28.75
ATOM   1640  CB  GLU A 224      38.476  39.348  86.606  1.00 29.18
ATOM   1641  CG  GLU A 224      37.470  39.099  87.741  1.00 29.05
ATOM   1642  CD  GLU A 224      37.335  40.348  88.557  1.00 29.41
ATOM   1643  OE1 GLU A 224      36.506  41.189  88.269  1.00 28.08
ATOM   1644  OE2 GLU A 224      38.060  40.487  89.516  1.00 31.14
ATOM   1645  N   PHE A 225      36.659  38.233  84.288  1.00 29.33
ATOM   1646  CA  PHE A 225      35.398  37.699  83.770  1.00 28.70
ATOM   1647  C   PHE A 225      35.551  36.354  83.061  1.00 31.53
ATOM   1648  O   PHE A 225      34.802  35.399  83.222  1.00 29.17
ATOM   1649  CB  PHE A 225      34.786  38.756  82.858  1.00 26.48
ATOM   1650  CG  PHE A 225      33.449  38.281  82.361  1.00 25.90
ATOM   1651  CD1 PHE A 225      32.361  38.258  83.250  1.00 28.71
ATOM   1652  CD2 PHE A 225      33.317  37.854  81.022  1.00 28.64
ATOM   1653  CE1 PHE A 225      31.129  37.758  82.817  1.00 29.98
ATOM   1654  CE2 PHE A 225      32.074  37.365  80.564  1.00 28.53
ATOM   1655  CZ  PHE A 225      30.998  37.309  81.479  1.00 30.02
ATOM   1656  N   ALA A 226      36.635  36.308  82.289  1.00 31.91
ATOM   1657  CA  ALA A 226      36.996  35.055  81.643  1.00 36.17
ATOM   1658  C   ALA A 226      37.178  33.829  82.536  1.00 40.94
ATOM   1659  O   ALA A 226      36.704  32.735  82.271  1.00 41.75
ATOM   1660  CB  ALA A 226      38.284  35.261  80.877  1.00 33.81
ATOM   1661  N   GLN A 227      37.883  34.081  83.647  1.00 44.91
ATOM   1662  CA  GLN A 227      38.067  32.931  84.543  1.00 49.32
ATOM   1663  C   GLN A 227      36.782  32.579  85.233  1.00 51.62
ATOM   1664  O   GLN A 227      36.396  31.467  85.528  1.00 50.02
ATOM   1665  CB  GLN A 227      39.239  33.150  85.499  1.00 50.80
ATOM   1666  CG  GLN A 227      40.441  33.937  84.900  1.00 59.92
ATOM   1667  CD  GLN A 227      40.812  33.681  83.397  1.00 68.68
ATOM   1668  OE1 GLN A 227      40.799  32.592  82.834  1.00 73.97
ATOM   1669  NE2 GLN A 227      41.221  34.764  82.726  1.00 65.71
ATOM   1670  N   HIS A 228      36.041  33.669  85.411  1.00 58.91
ATOM   1671  CA  HIS A 228      34.687  33.501  85.925  1.00 65.53
ATOM   1672  C   HIS A 228      33.816  32.519  85.143  1.00 68.04
ATOM   1673  O   HIS A 228      33.014  31.764  85.662  1.00 67.46
ATOM   1674  CB  HIS A 228      34.091  34.903  86.045  1.00 68.11
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ATOM   1675  CG  HIS A 228      32.632  34.780  86.283  1.00 71.87
ATOM   1676  ND1 HIS A 228      31.729  34.968  85.313  1.00 74.51
ATOM   1677  CD2 HIS A 228      32.007  34.363  87.458  1.00 75.53
ATOM   1678  CE1 HIS A 228      30.511  34.660  85.852  1.00 78.68
ATOM   1679  NE2 HIS A 228      30.682  34.288  87.171  1.00 80.02
ATOM   1680  N   GLN A 229      34.061  32.545  83.846  1.00 74.45
ATOM   1681  CA  GLN A 229      33.306  31.659  82.963  1.00 81.06
ATOM   1682  C   GLN A 229      33.569  30.149  83.028  1.00 85.59
ATOM   1683  O   GLN A 229      33.123  29.427  82.135  1.00 86.52
ATOM   1684  CB  GLN A 229      33.477  32.181  81.530  1.00 81.47
ATOM   1685  CG  GLN A 229      33.002  33.631  81.335  1.00 81.55
ATOM   1686  CD  GLN A 229      31.488  33.690  81.352  1.00 83.81
ATOM   1687  OE1 GLN A 229      30.804  33.832  82.355  1.00 82.35
ATOM   1688  NE2 GLN A 229      30.950  33.588  80.141  1.00 86.80
ATOM   1689  N   LYS A 230      34.317  29.749  84.086  1.00 91.08
ATOM   1690  CA  LYS A 230      34.965  28.444  84.325  1.00 95.45
ATOM   1691  CB  LYS A 230      33.976  27.245  84.052  1.00 97.51
ATOM   1692  CG  LYS A 230      34.256  26.053  83.073  1.00 98.30
ATOM   1693  CD  LYS A 230      34.035  26.121  81.534  1.00 98.30
ATOM   1694  CE  LYS A 230      34.810  27.172  80.713  1.00100.00
ATOM   1695  NZ  LYS A 230      36.244  27.239  81.067  1.00100.00
ATOM   1696  C   LYS A 230      36.409  28.279  83.743  1.00 97.06
ATOM   1697  OCT1 LYS A 230      36.876  29.108  82.942  1.00 95.86
ATOM   1698  OCT2 LYS A 230      37.052  27.241  83.957  1.00 99.89
ATOM   1935  S   SO4 S 231      22.561  63.872  89.148  1.00 45.29
ATOM   1936  O1  SO4 S 231      21.748  62.858  88.279  1.00 50.45
ATOM   1937  O2  SO4 S 231      21.648  64.707  90.036  1.00 51.74
ATOM   1938  O3  SO4 S 231      23.551  63.095  90.035  1.00 49.75
ATOM   1939  O4  SO4 S 231      23.260  64.912  88.285  1.00 44.08
ATOM      1  O   HOH W 232      10.522  63.513  85.670  1.00 17.86
ATOM      2  O   HOH W 233      34.116  63.633  80.578  1.00 20.45
ATOM      3  O   HOH W 234       7.928  61.775  88.229  1.00 15.62
ATOM      4  O   HOH W 235      10.374  64.545  82.597  1.00 14.58
ATOM      5  O   HOH W 236      15.375  75.641  85.508  1.00 22.07
ATOM      6  O   HOH W 237      20.773  44.507  86.785  1.00 18.67
ATOM      7  O   HOH W 238      32.701  49.912  75.935  1.00 15.79
ATOM      8  O   HOH W 239      21.979  72.096  84.493  1.00 19.08
ATOM      9  O   HOH W 240      13.158  73.905  82.705  1.00 27.34
ATOM     10  O   HOH W 241      14.358  71.880  73.410  1.00 26.83
ATOM     11  O   HOH W 242       5.537  80.043  74.802  1.00 23.33
ATOM     12  O   HOH W 243      36.136  62.604  78.407  1.00 23.19
ATOM     13  O   HOH W 244      30.393  53.028  87.579  1.00 19.02
ATOM     14  O   HOH W 245      28.532  49.107  93.252  1.00 21.32
ATOM     15  O   HOH W 246      24.657  73.146  75.882  1.00 20.92
ATOM     16  O   HOH W 247      10.080  55.567  81.848  1.00 33.80
ATOM     17  O   HOH W 248      29.907  52.840  73.379  1.00 22.59
ATOM     18  O   HOH W 249      38.583  48.054  74.575  1.00 24.10
ATOM     19  O   HOH W 250      29.465  68.020  86.676  1.00 32.30
ATOM     20  O   HOH W 251      12.847  73.680  85.460  1.00 40.76
ATOM     21  O   HOH W 252       5.516  59.770  95.129  1.00 40.84
ATOM     22  O   HOH W 253      42.504  47.354  77.319  1.00 30.77
ATOM     23  O   HOH W 254      13.495  75.378  74.412  1.00 22.57
ATOM     24  O   HOH W 255      17.100  76.564  77.737  1.00 30.00
ATOM     25  O   HOH W 256      33.508  40.103 102.712  1.00 26.49
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ATOM	26	O	HOH W 257	20.825	55.648	81.278	1.00	20.11
ATOM	27	O	HOH W 258	19.730	61.701	89.970	1.00	23.10
ATOM	28	O	HOH W 259	4.363	74.520	80.720	1.00	33.74
ATOM	29	O	HOH W 260	31.490	42.656	98.480	1.00	34.19
ATOM	30	O	HOH W 261	6.696	75.130	78.477	1.00	15.66
ATOM	31	O	HOH W 262	10.667	67.023	75.103	1.00	38.86
ATOM	32	O	HOH W 263	8.252	64.433	92.307	1.00	23.15
ATOM	33	O	HOH W 264	41.924	51.223	74.247	1.00	30.19
ATOM	34	O	HOH W 265	1.437	67.705	89.398	1.00	39.48
ATOM	35	O	HOH W 266	4.055	66.946	91.467	1.00	29.22
ATOM	36	O	HOH W 267	3.092	69.112	84.950	1.00	25.58
ATOM	37	O	HOH W 268	9.537	59.065	79.795	1.00	30.90
ATOM	38	O	HOH W 269	9.306	83.197	79.638	1.00	44.19
ATOM	39	O	HOH W 270	34.786	41.166	75.522	1.00	32.98
ATOM	40	O	HOH W 271	28.084	37.193	84.163	1.00	30.43
ATOM	41	O	HOH W 272	40.742	49.227	76.024	1.00	21.82
ATOM	42	O	HOH W 273	35.074	40.712	85.668	1.00	29.87
ATOM	43	O	HOH W 274	30.318	45.526	96.384	1.00	35.57
ATOM	44	O	HOH W 275	31.493	69.162	80.850	1.00	19.51
ATOM	45	O	HOH W 276	42.914	61.700	76.016	1.00	28.69
ATOM	46	O	HOH W 277	34.422	64.714	92.625	1.00	38.81
ATOM	47	O	HOH W 278	13.405	78.374	80.916	1.00	25.22
ATOM	48	O	HOH W 279	44.634	57.811	84.433	1.00	31.73
ATOM	49	O	HOH W 280	44.303	60.992	82.740	1.00	28.14
ATOM	50	O	HOH W 281	32.596	51.432	73.247	1.00	22.63
ATOM	51	O	HOH W 282	22.182	40.126	75.125	1.00	27.50
ATOM	52	O	HOH W 283	18.482	55.362	89.100	1.00	21.25
ATOM	53	O	HOH W 284	36.960	42.360	74.192	1.00	28.88
ATOM	54	O	HOH W 285	35.881	48.845	94.047	1.00	26.90
ATOM	55	O	HOH W 286	26.212	59.698	94.760	1.00	23.37
ATOM	56	O	HOH W 287	29.246	44.303	73.369	1.00	40.38
ATOM	57	O	HOH W 288	27.356	35.947	80.422	1.00	31.74
ATOM	58	O	HOH W 289	40.482	45.029	76.766	1.00	30.88
ATOM	59	O	HOH W 290	24.864	58.724	91.112	1.00	25.30
ATOM	60	O	HOH W 291	28.560	61.547	91.755	1.00	39.37
ATOM	61	O	HOH W 292	27.888	63.113	90.252	1.00	40.28
ATOM	62	O	HOH W 293	31.069	41.023	103.435	1.00	38.13
ATOM	63	O	HOH W 294	5.144	47.860	86.978	1.00	37.63
ATOM	64	O	HOH W 295	29.373	52.425	90.409	1.00	21.69
ATOM	65	O	HOH W 296	41.571	51.401	87.864	1.00	31.72
ATOM	66	O	HOH W 297	35.633	56.807	101.396	1.00	42.27
ATOM	67	O	HOH W 298	35.257	40.157	78.063	1.00	30.17
ATOM	68	O	HOH W 299	33.734	71.189	79.910	1.00	32.64
ATOM	69	O	HOH W 300	17.659	69.593	75.158	1.00	46.73
ATOM	70	O	HOH W 301	17.005	72.932	72.774	1.00	33.93
ATOM	71	O	HOH W 302	15.769	48.059	85.107	1.00	24.21
ATOM	72	O	HOH W 303	15.023	64.697	75.333	1.00	39.99
ATOM	73	O	HOH W 304	13.546	67.305	74.469	1.00	38.11
ATOM	74	O	HOH W 305	30.044	75.863	82.738	1.00	29.02
ATOM	75	O	HOH W 306	5.253	66.383	98.323	1.00	61.09
ATOM	76	O	HOH W 307	25.914	72.829	89.073	1.00	48.08
ATOM	77	O	HOH W 308	38.474	67.620	76.050	1.00	32.88
ATOM	78	O	HOH W 309	34.101	41.534	100.215	1.00	39.54
ATOM	79	O	HOH W 310	29.974	37.419	76.650	1.00	39.99

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ATOM     80  O   HOH W 311      17.829  44.406  81.773  1.00 29.29
ATOM     81  O   HOH W 312      17.766  66.478  75.705  1.00 34.51
ATOM     82  O   HOH W 313      35.983  70.225  78.152  1.00 38.85
ATOM     83  O   HOH W 314      18.063  58.644  75.592  1.00 33.24
ATOM     84  O   HOH W 315      17.740  45.824  75.692  1.00 31.29
ATOM     85  O   HOH W 316      21.442  55.668 101.498  1.00 30.06
ATOM     86  O   HOH W 317      30.660  37.639 105.501  1.00 46.34
ATOM     87  O   HOH W 318      28.143  47.582  99.410  1.00 71.00
ATOM     88  O   HOH W 319      11.398  65.394  76.821  1.00 34.86
ATOM     89  O   HOH W 320      31.737  45.760  98.744  1.00 38.11
ATOM     90  O   HOH W 321      16.084  45.559  87.137  1.00 43.68
ATOM     91  O   HOH W 322      36.498  37.962  78.989  1.00 35.45
ATOM     92  O   HOH W 323      41.868  42.172  76.980  1.00 56.04
ATOM     93  O   HOH W 324      44.704  68.004  76.606  1.00 73.28
ATOM     94  O   HOH W 325      30.214  44.935 101.119  1.00 28.63
ATOM     95  O   HOH W 326      43.719  69.244  83.004  1.00 32.20
ATOM     96  O   HOH W 327       7.992  54.768  93.490  1.00 36.05
ATOM     97  O   HOH W 328      11.059  49.604  75.476  1.00 43.80
ATOM     98  O   HOH W 329      17.730  37.202  79.516  1.00 44.41
ATOM     99  O   HOH W 330      14.170  59.796  74.913  1.00 70.26
ATOM    100  O   HOH W 331      28.648  70.326  88.645  1.00 34.35
ATOM    101  O   HOH W 332      16.146  57.197  73.492  1.00 49.27
ATOM    102  O   HOH W 333      11.086  52.502  82.116  1.00 39.47
ATOM    103  O   HOH W 334      15.950  60.744  73.392  1.00 63.16
ATOM    104  O   HOH W 335      23.809  74.443  89.142  1.00 63.73
ATOM    105  O   HOH W 336      43.077  70.945  86.543  1.00 41.77
ATOM    106  O   HOH W 337      44.625  68.578  85.466  1.00 42.53
ATOM    107  O   HOH W 338      38.003  70.941  79.707  1.00 47.97
ATOM    108  O   HOH W 339      42.635  39.826  86.317  1.00 39.90
ATOM    109  O   HOH W 340      28.158  51.028  97.893  1.00 35.28
ATOM    110  O   HOH W 341      34.562  57.666  98.193  1.00 56.42
ATOM    111  O   HOH W 342      23.659  34.535  79.197  1.00 84.39
ATOM    112  O   HOH W 343      10.337  58.458  76.704  1.00 45.85
ATOM    113  O   HOH W 344      32.164  75.101  85.461  1.00 54.21
ATOM    114  O   HOH W 345      32.930  38.410  86.586  1.00 43.15
ATOM    115  O   HOH W 346      32.310  36.987 102.558  1.00 47.71
ATOM    116  O   HOH W 347      11.163  49.101  82.634  1.00 84.37
ATOM    117  O   HOH W 348      34.268  69.634  83.019  1.00 47.39
ATOM    118  O   HOH W 349      31.352  37.085  89.579  1.00 74.88
ATOM    119  O   HOH W 350      29.118  56.986  95.860  1.00 34.59
ATOM    120  O   HOH W 351       1.634  70.786  81.659  1.00 41.89
ATOM    121  O   HOH W 352       2.044  71.714  85.736  1.00 37.84
ATOM    122  O   HOH W 353      16.219  75.511  74.471  1.00 44.53
ATOM    123  O   HOH W 354      24.035  45.705  97.204  1.00 48.11
ATOM    124  O   HOH W 355      17.939  77.382  82.853  1.00 65.65
ATOM    125  O   HOH W 356      12.504  76.991  70.634  1.00 50.43
ATOM    126  O   HOH W 357      16.951  78.295  74.889  1.00 47.02
ATOM    127  O   HOH W 358      15.777  75.404  81.566  1.00 33.68
ATOM    128  O   HOH W 359      37.401  72.376  82.831  1.00 50.52
ATOM    129  O   HOH W 360      14.060  44.359  88.918  1.00 80.84
ATOM    130  O   HOH W 361      32.619  76.123  75.757  1.00 42.84
ATOM    131  O   HOH W 362      21.836  66.226  94.339  1.00 63.40
ATOM    132  O   HOH W 363      16.011  46.526  82.837  1.00 38.42
ATOM    133  O   HOH W 364       7.716  57.886  82.470  1.00 50.22
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ATOM    134  O   HOH W 365      41.813  72.155  81.960  1.00 76.60
ATOM    135  O   HOH W 366       5.810  63.614  94.440  1.00 41.72
ATOM    136  O   HOH W 367      22.833  66.006  98.308  1.00 65.79
ATOM    137  O   HOH W 368      21.384  36.791  76.692  1.00 59.29
ATOM    138  O   HOH W 369      38.765  52.950  92.219  1.00 27.87
ATOM    139  O   HOH W 370      46.430  68.991  81.609  1.00 70.52
ATOM    140  O   HOH W 371      36.973  69.709  83.153  1.00 33.81
ATOM    141  O   HOH W 372      22.238  43.716  92.825  1.00 44.49
ATOM    142  O   HOH W 373      23.096  80.189  77.308  1.00 50.73
ATOM    143  O   HOH W 374       2.790  53.932  81.478  1.00 50.22
ATOM    144  O   HOH W 375       3.292  64.768  94.055  1.00 46.64
ATOM    145  O   HOH W 376      26.937  79.257  75.755  1.00 47.21
ATOM    146  O   HOH W 377      45.046  50.594  85.873  1.00 46.10
ATOM    147  O   HOH W 378      24.988  68.312  90.158  1.00 39.03
ATOM    148  O   HOH W 379       2.045  61.203  93.643  1.00 49.73
ATOM    149  O   HOH W 380      44.273  56.110  87.700  1.00 46.74
ATOM    150  O   HOH W 381      26.747  76.462  73.043  1.00 50.91
ATOM    151  O   HOH W 382      40.545  70.889  76.918  1.00 68.80
ATOM    152  O   HOH W 383      25.523  80.486  83.807  1.00 67.90
ATOM    153  O   HOH W 384      40.972  36.296  87.372  1.00 66.49
ATOM    154  O   HOH W 385      12.617  56.710  77.567  1.00 44.81
ATOM    155  O   HOH W 386      44.460  48.054  74.082  1.00 41.02
ATOM    156  O   HOH W 387      35.781  73.896  86.117  1.00 38.14
ATOM    157  O   HOH W 388      21.625  80.398  81.815  1.00 47.96
ATOM    158  O   HOH W 389      46.628  56.635  82.977  1.00 50.89
ATOM    159  O   HOH W 390      12.308  51.573  78.083  1.00 64.92
ATOM    160  O   HOH W 391      30.773  39.420  87.798  1.00 55.92
ATOM    161  O   HOH W 392      26.088  65.110  89.923  1.00 44.80
ATOM    162  O   HOH W 393      10.719  70.886  96.928  1.00 48.46
ATOM    163  O   HOH W 394      12.474  47.243  84.457  1.00 53.08
ATOM    164  O   HOH W 395      24.296  71.312  91.828  1.00 48.39
ATOM    165  O   HOH W 396       6.459  50.108  83.133  1.00 57.82
ATOM    166  O   HOH W 397      42.423  66.213  75.196  1.00 34.97
ATOM    167  O   HOH W 398      29.045  53.518 101.769  1.00 46.59
ATOM    168  O   HOH W 399      27.195  39.655 105.406  1.00 41.59
ATOM    169  O   HOH W 400       6.834  56.385  96.211  1.00 45.81
ATOM    170  O   HOH W 401      47.957  50.138  78.280  1.00 43.60
ATOM    171  O   HOH W 402      23.330  36.461  72.787  1.00 54.41
ATOM    172  O   HOH W 403      29.051  79.533  81.900  1.00 78.87
ATOM    173  O   HOH W 404      46.670  55.026  74.340  1.00 68.61
ATOM    174  O   HOH W 405      28.985  78.746  85.840  1.00 75.16
ATOM    175  O   HOH W 406      32.117  68.589  73.365  1.00 42.10
ATOM    176  O   HOH W 407      48.677  52.842  75.727  1.00 66.77
ATOM    177  O   HOH W 408      29.185  36.245  72.017  1.00 75.24
ATOM    178  O   HOH W 409      37.168  67.596  97.670  1.00 38.24
ATOM    179  O   HOH W 410      11.986  77.352  92.370  1.00 35.94
ATOM    180  O   HOH W 411      39.548  63.174  98.280  1.00 39.58
ATOM    181  O   HOH W 412      30.500  79.967  79.292  1.00 62.87
ATOM    182  O   HOH W 413      18.003  41.205  83.764  1.00 64.48
ATOM    183  O   HOH W 414      34.455  37.242  89.080  1.00 52.58
ATOM    184  O   HOH W 415      47.074  60.938  83.746  1.00 66.98
ATOM    185  O   HOH W 416      10.880  54.535  78.559  1.00 45.29
ATOM    186  O   HOH W 417      30.230  76.830  74.341  1.00 71.09
ATOM    187  O   HOH W 418      12.118  81.147  79.341  1.00 49.66
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ATOM    188  O   HOH W 419      32.095  76.945  80.548  1.00 63.23
ATOM    189  O   HOH W 420      -0.301  68.264  84.539  1.00 48.83
ATOM    190  O   HOH W 421      10.822  64.227 102.313  1.00 81.32
ATOM    191  O   HOH W 422      23.374  42.555 101.170  1.00 42.91
ATOM    192  O   HOH W 423      20.016  59.713  74.793  1.00 38.45
ATOM    193  O   HOH W 424      15.833  78.412  79.495  1.00 56.01
ATOM    194  O   HOH W 425      43.534  35.850  84.957  1.00 63.16
ATOM    195  O   HOH W 426      11.933  68.018  98.874  1.00 52.15
ATOM    196  O   HOH W 427      20.777  37.368  85.962  1.00 57.71
ATOM    197  O   HOH W 428      22.392  36.632  89.560  1.00 68.43
ATOM    198  O   HOH W 429      29.340  37.487 101.980  1.00 74.20
ATOM    199  O   HOH W 430      23.237  39.294  91.878  1.00 74.07
ATOM    200  O   HOH W 431      13.654  75.325  94.697  1.00 73.83
ATOM    201  O   HOH W 432      27.904  38.307  96.631  1.00 57.14
ATOM    202  O   HOH W 433      44.213  59.909  79.188  1.00 37.71
ATOM    203  O   HOH W 434       2.129  75.408  79.755  1.00 64.17
ATOM    204  O   HOH W 435      13.993  43.469  84.483  1.00 59.78
ATOM    205  O   HOH W 436      31.644  55.529  99.951  1.00 58.81
ATOM    206  O   HOH W 437       9.462  82.415  76.470  1.00 48.44
ATOM    207  O   HOH W 438      21.813  58.761  98.061  1.00 60.37
ATOM    208  O   HOH W 439      22.202  59.533  93.382  1.00 43.39
ATOM    209  O   HOH W 440      18.118  43.497  86.455  1.00 46.86
ATOM    210  O   HOH W 441      13.762  54.340 105.466  1.00 57.78
ATOM    211  O   HOH W 442      33.277  73.931  83.853  1.00 56.73
ATOM    212  O   HOH W 443      34.442  68.648  90.744  1.00 27.90
ATOM    213  O   HOH W 444      30.640  67.899  91.831  1.00 53.48
ATOM    214  O   HOH W 445      40.813  44.217  74.058  1.00 53.35
ATOM    215  O   HOH W 446      33.012  71.334  90.213  1.00 53.98
ATOM    216  O   HOH W 447      25.130  57.928 101.293  1.00 38.97
ATOM    217  O   HOH W 448       7.584  82.067  74.163  1.00 26.55
ATOM    218  O   HOH W 449      42.214  40.521  78.980  1.00 37.75
ATOM    219  O   HOH W 450       8.915  57.776 101.115  1.00 50.37
ATOM    220  O   HOH W 451      15.963  42.582  79.699  1.00 71.24
ATOM    221  O   HOH W 452      23.011  77.967  75.363  1.00 64.04
ATOM    222  O   HOH W 453      36.910  35.452  88.469  1.00 70.47
ATOM    223  O   HOH W 454      37.814  55.271  99.966  1.00 54.36
ATOM    224  O   HOH W 455      26.721  58.439  99.230  1.00 86.46
ATOM    225  O   HOH W 456      16.108  40.093  81.126  1.00 98.28
ATOM    226  O   HOH W 457      27.800  35.543  96.536  1.00 63.56
ATOM    227  O   HOH W 458       5.859  51.318  95.801  1.00 68.96
ATOM    228  O   HOH W 459       7.841  51.875  96.622  1.00 64.76
ATOM    229  O   HOH W 460      28.280  66.535  89.122  1.00 73.27
ATOM    230  O   HOH W 461      13.943  46.268  81.680  1.00 56.20
ATOM    231  O   HOH W 462      14.681  69.220  73.344  1.00 83.46
ATOM    232  O   HOH W 463      30.388  71.379  89.815  1.00 60.45
ATOM    233  O   HOH W 464       8.062  56.915  75.809  1.00 59.76
ATOM    234  O   HOH W 465      30.104  41.907 101.688  1.00 61.89
ATOM    235  O   HOH W 466       4.988  49.407  95.471  1.00 61.48
ATOM    236  O   HOH W 467       8.747  53.997  77.187  1.00 79.74
END
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【図４６】

A72F(s)　5'-CA-GAC-CTG-GCC-TTT-GGC-GAT-GTG-GC-3'

A72F(as)　3'-GT-CTG-GAC-CGG-AAA-CCG-CTA-CAC-CG-5'

D　L　A　F_{72}　G　D　V

A72E(s)　5'-CA-GAC-CTG-GCC-GAA-GGC-GAT-GTG-GC-3'

A72E(as)　3'-GT-CTG-GAC-CGG-CTT-CCG-CTA-CAC-CG-5'

D　L　A　E_{72}　G　D　V

【図４７】

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I103D(s)  5'-TG-ACC-AAT-ATG-GAC-GAG-GAC-GCC-GG-3'
I103D(as) 3'-AC-TGG-TTA-TAC-CTG-CTC-CTG-CGG-CC-5'
                T   N   M   D103 E   D   A
T153N(s)  5'-GG-CAT-ACC-TCT-AAC-GGC-TGG-GCT-AC-3'
T153N(as) 3'-CC-GTA-TGG-AGA-TTG-CCG-ACC-CGA-TG-5'
                H   T   S   N153 G   W   A
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【図４８】

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L140F(s)  5'-AC-CAG-GAC-AAA-TTC-TCC-AAA-AAT-GG-3'
L140F(as) 3'-TG-GTC-CTG-TTT-AAG-AGG-TTT-TTA-CC-5'
                Q   D   K   F140 S   K   N
L140K(s)  5'-AC-CAG-GAC-AAA-AAA-TCC-AAA-AAT-GG-3'
L140K(as) 3'-TG-GTC-CTG-TTT-TTT-AGG-TTT-TTA-CC-5'
                Q   D   K   K140 S   K   N
L140E(s)  5'-AC-CAG-GAC-AAA-GAA-TCC-AAA-AAT-GG-3'
L140E(as) 3'-TG-GTC-CTG-TTT-CTT-AGG-TTT-TTA-CC-5'
                Q   D   K   E140 S   K   N
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【手続補正書】
【提出日】平成１２年９月１４日（２０００．９．１４）
【手続補正１】
【補正対象書類名】明細書
【補正対象項目名】００５０
【補正方法】変更
【補正内容】
【００５０】実施例２０　エンテロバクター・アエロゲネス IFO 12010 由来野生型酸性ホスファターゼの精製とＮ末端アミノ酸配列の決定
特開平10-201481号公報の実施例24記載のエシェリヒア・コリJM109/pENP110の培養菌体からエンテロバクター・アエロゲネスIFO12010由来の酸性ホスファターゼを精製してＮ末端アミノ酸配列を決定し、成熟蛋白質のアミノ酸配列を決定した。エシェリヒア・コリ JM109/pENP110はエンテロバクター・アエロゲネスIFO12010由来の酸性ホスファターゼ遺伝子をエシェリヒア・コリJM109株に導入した菌で、該酸性ホスファターゼを生産する。該酸性ホスファターゼ遺伝子の塩基配列より予想される前駆体蛋白質のアミノ酸配列は配列表の配列番号１０に示される配列に相当する。配列番号10に示すアミノ酸配列は、L61Q/A63Q/E64A/N67D/S69A/G72D/T133K/E134D/I151T変異型EA-APのアミノ酸配列である。ペプトン１g/dl、酵母エキス0.5g/dl及び食塩１g/dlを含有する栄養培地（pH7.0）50mlを500ml坂口フラスコに入れ、120℃にて20分間加熱殺菌した。これにエシェリヒア・コリ JM109/pENP110を一白金耳接種し、30℃で16時間振とう培養した。培養液から遠心分離により菌体を回収した菌体を100 ｍｌの100mMリン酸カリウムバッファー（pH7.0）に懸濁し、４℃で20分間超音波処理を行い菌体を破砕した。処理液を遠心分離して不溶性画分を除き、無細胞抽出液を調製した。この無細胞抽出液に30%飽和となるように硫酸アンモニウムを添加した。遠心分離により生成した沈殿を除去した後、上清液に60%飽和となるように硫酸アンモニウムを追加添加した。生成した沈殿を遠心分離により回収し、100mMリン酸カリウムバッファーに溶解した。この粗酵素液を100mMリン酸カリウムバッファー（pH7.0）500mlに対し3回透析した後、20mMリン酸カリウムバッファー（pH7.0）で平衡化したDEAE-トヨパール650Mカラム（φ3.0×10.0cm）にチャージし、20mMリン酸カリウムバッファー（pH7.0）で洗浄した。リン酸転移活性は素通り画分にあったので、当該画分を回収した。 この活性画分に35%飽和となるように硫酸アンモニウムを添加し、これを35%飽和硫酸アンモニウムを含む20mMリン酸カリウムバッファー（pH7.0）で平衡化したブチルトヨパールカラム（φ3.0×7.0cm）に吸着させた。これを35%飽和から20%飽和リン酸カリウムバッファー（pH7.0）の直線的な濃度勾配で溶出した。活性画

分を集め、10mMリン酸カリウムバッファー（pH6.0）１Ｌに対し透析した後、10mMリン酸カリウムバッファー（pH6.0）で平衡化したCM-トヨパール カラム（φ3.0×7.0cm）に吸着させた。これを0mMから300mM 塩化カリウムを含むリン酸カリウムバッファー（pH6.0）の直線的な濃度勾配で溶出した。この活性画分を集めた。以上の操作によって、リン酸転移活性を示す酵素を無細胞抽出液より最終的に約16%の回収率で約5倍に精製した。この酵素標品は、ＳＤＳ－ポリアクリルアミド電気泳動において均一であった。本精製酵素をＤＩＴＣ　メンブレン〔ミリゲン／バイオサーチ（Milligen/Biosearch）社製〕に吸着させ、Prosequencer　6625（ミリゲン／バイオサーチ社製）を用いてＮ末端のアミノ酸配列を決定したところ配列表の配列番号９８に示した５残基のＮ末端のアミノ酸配列が決定された。精製酵素のＮ末端は配列表の配列番号１０の配列の21番目のアラニン残基から開始していたため、配列表の配列番号１０に示されるアミノ酸配列は前駆体蛋白質の配列であり、１番目のメチオニン残基から20番目のフェニルアラニン残基までのペプチドは翻訳後に除去されるものと考えられた。この結果より成熟体蛋白質のアミノ酸配列は配列表の配列番号１０に示される配列中、アミノ酸番号１～２２８に示される配列に相当する。

【手続補正２】
【補正対象書類名】明細書
【補正対象項目名】００６２
【補正方法】変更
【補正内容】
【００６２】実施例２３　グアノシンに対する親和性の向上したエンテロバクター・アエロゲネス IFO 12010由来新規変異型酸性ホスファターゼ遺伝子導入菌によるグアノシンのリン酸化反応
それぞれの変異型酸性ホスファターゼ遺伝子を含むプラスミドを導入したエシェリヒア・コリ　ＪＭ109／pENP180、エシェリヒア・コリＪＭ109／pENP320、エシェリヒア・コリＪＭ109／pENP340、エシェリヒア・コリＪＭ109／pENP410、エシェリヒア・コリＪＭ109／pENP510、及びエシェリヒア・コリＪＭ109／pENP520をアンピシリン100μg/ml及びＩＰＴＧ1mMを含むＬ培地50mｌに接種し、37℃で16時間培養した。ピロリン酸10g/dl、及び特願平12-189226号明細書の実施例１と同様に調製した粉砕グアノシン6.6g/dlを100mM酢酸バッファー（pH4.5）に溶解し、これにそれぞれの菌体を乾燥菌体重量で100mg/dlとなるように添加し、pHを4.5に維持しながら、35℃で12時間反応させた。生成した5′－グアニル酸の量を表１７に示した。表１７に示すように変異型酵素を導入した菌はいずれも親株であるエシェリヒア・コリ　ＪＭ109／pENP180に比べて生産性が向上し、高い収率で5′－グアニル酸を生成蓄積した。

フロントページの続き

(51) Int.Cl.[7]		識別記号	FI		テーマコード（参考）
Ｃ１２Ｎ	9/12		Ｃ１２Ｐ	19/38	
	9/16		Ｃ１２Ｒ	1:01)	
Ｃ１２Ｐ	19/38			1:185)	
//(Ｃ１２Ｎ	15/09	ＺＮＡ		1:22)	
Ｃ１２Ｒ	1:01)		(Ｃ１２Ｐ	19/38	
(Ｃ１２Ｎ	15/09	ＺＮＡ	Ｃ１２Ｒ	1:19)	
Ｃ１２Ｒ	1:185)		Ｃ１２Ｎ	15/00	ＺＮＡＡ
(Ｃ１２Ｎ	15/09	ＺＮＡ		5/00	Ａ
Ｃ１２Ｒ	1:22)		Ｃ１２Ｒ	1:01)	
(Ｃ１２Ｐ	19/38			1:185)	
Ｃ１２Ｒ	1:19)			1:22)	

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